THE

EXTERNAL SECRETION

OF THE

PANCREAS

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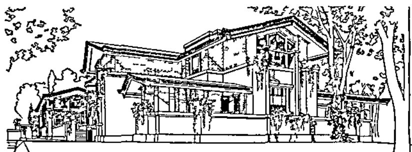
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PREFACE

If I had been writing a monograph for physicians some twenty years ago I should have felt obliged to confine myself to the so called practical aspects of the subject, at the present time I feel no such compulsion It is my observation that modern physicians are about as much interested in the sciences that are basic to their practice as are those who spend their whole time in teaching and research It is unfortunate that this interest is often frustrated through lack of the time required to consult original sources If this discussion helps in a small way to improve that situation it will have served one of its purposes Another objective has been to provide a convenient guide to source material for students and teachers of physiology

In a work of this length it is, of course, impossible to cite all the literature or even a major part of it References have been selected for their historical significance, their usefulness, and, too often perhaps, their availability Articles that have been published in more than one language have been cited, by preference, in the language most likely to be familiar to the reader This applies particularly to articles originally published in the Russian language and later reviewed or published in translation in French or German journals

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Through the kindness of several colleagues I have been able to include in the text some observations and opinions that have not yet found their way into the published literature I take this occasion to express my gratitude to Drs Horace W Davenport, M H F Friedman, E S Nasset, I J Pincus, V Brown Scott, and Wm J Snape for permission to use their unpublished contributions I am also indebted to Drs N A Michels and A J Ramsay for their indispensable help in writing the chapter on Morphology and to Dr M H F Friedman for his assistance in preparing the section on Secretin and for reading and correcting the entire manuscript Acknowledgement is also due to Dr S A Kornarov for many valuable criticisms and suggestions

Prof B P Babkin has been kind enough to read and criticize several of the chapters and has made many helpful suggestions His interest in our work has been a constant source of stimulation and encouragement I am further indebted to Prof Babkin, as every writer in this field must be, in that his publications have served as guides to the early literature and as sources for much of the subject matter

J E T

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Chapter I

MORPHOLOGY

THE pancreas is an elongated gland which extends from the duodenum obliquely upward behind the stomach across the posterior abdominal wall to the spleen, at the level of the first and second lumbar vertebrae For descriptive purposes it is divided into a head, body and tail The parts are not separated by any well-defined markings but form a continuous retroperitoneal mass firmly attached to the posterior abdominal wall The most massive portion of the gland is the head It is flattened dorsoventrally and lies within the concavity of the duodenum, to which it is attached by numerous blood vessels the pancreatic ducts and loose connective tissue The uncinate process is a hook-like medial projection of the lower part of the head The body of the pancreas makes up the major portion of the remainder of the gland It is somewhat prismatic in shape and presents an anterior, a posterior and an inferior surface The tail is the pointed tongue-like left end of the gland which lies in contact with the spleen

The terms used to describe the human pancreas are applicable also to the pancreas of the dog and cat The principal difference from the human is the greater mobility of the pancreas in these animals, particularly in the region of the head which lies within the duodenal mesentery The uncinate process is well developed and parallels the descending portion of the duodenum In the rabbit and other rodents the pancreas is rather diffusely distributed in the mesentery of the upper intestine

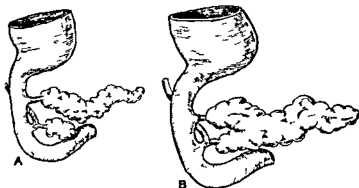


Figure 1

Pancreas of the human embryo A Fifth week B Seventh week (From Reinhoff and Pickrell (2) Archives of Surgery)

with the liver bud the duct of Wirsung in the adult enters the duodenum in close association with the common bile duct That portion of the duct of the dorsal rudiment which lies between the duodenum and the point of anastomosis with the ventral duct is normally retarded in its subsequent development and becomes the accessory duct (ductus pancreaticus accessorius or duct of Santorini)

The duodenal end of the accessory duct was found not to be patent in 24 per cent of 250 cases examined by Reinhoff and Pickrell (2) Probably in a majority of instances, according to their findings, it drains into the major duct rather than into the duodenum These authors give the following frequency of other anomalies of the pancreatic ducts failure of the ducts to anastomose within the pancreas, 15 per cent, accessory duct larger than the "principal" duct, 6 61 per cent, no accessory duct 0 737 per cent In calculating their percentages they included with their data

The following anomalies of the pancreas have been described (1) as of rather frequent occurrence 1 The uncinate process may develop as a separate structure which then becomes a "lesser pancreas" 2 Glandular tissue from the head of the pancreas may completely surround the descending duodenum forming an "annular pancreas" 3 Pancreatic tissue may develop in unusual locations, chiefly in the wall of the stomach or the jejunum, resulting in an "accessory pancreas"

THE PANCREATIC DUCTS

Embryology The peculiarities of the duct system of the pancreas are intelligible only when considered in relation to the embryologic development The gland develops from two outgrowths of the primitive gut one arising from the ventral and the other from the dorsal wall immediately below the primitive stomach The ventral rudiment (at times double) appears to develop in common with the embryonic liver bud whereas the dorsal rudiment develops directly from the gut as a single outpocketing As a result of rotation of the gut and its mesenteries and the growth of the rudiments themselves the two portions of the pancreas come into contact at the left of the duodenum and eventually fuse (Fig 1, B) The dorsal pancreatic bud grows more rapidly than the ventral, producing some of the head of the gland, its body, and its tail, the main portion of the head is contributed by the ventral bud The duct of the ventral pancreas joins the ducts of the dorsal portion somewhere along the course of the latter, usually near its origin and the two subsequently form a continuous channel which constitutes the major pancreatic duct (ductus pancreaticus or duct of Wirsung)

In keeping with the fact that the proximal portion of this duct (ventral bud) was developed in conjunction



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the results of previous studies by Opie (3) and others The problem was also studied by Simkins (4) The most frequent arrangement of the ducts as found by Reinhoff and Pickrell is illustrated in Fig 2 A

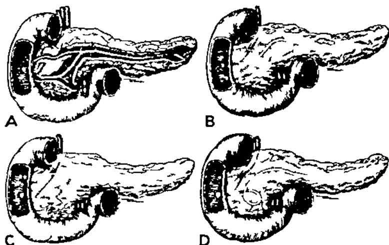


Figure 2

A The most frequent arrangement of the pancreatic ducts B Specimen with three papillae C Dissected specimen of an adult pancreas showing an embryonic type of duct system in which the accessory duct carries most of the secretion D Dissected specimen of an adult pancreas showing an unusual loop configuration of the main pancreatic duct (From Reinhoff and Pickrell (2) Archives of Surgery)

A matter of considerable clinical interest is the relation of the pancreatic duct to the common bile duct at the point of their common entrance into the duodenum They may enter separately or form a conjoined bile and pancreatic duct which, as it passes through the duodenum, dilates to form the ampulla of

Vater which opens into the duodenal papilla In the series studied by Reinhoff and Pickrell (2) an ampulla was present in only 81 of their 250 cases Combining their data with those previously published they arrived at a figure of 46 per cent for all cases reported In the remaining 53 per cent the ducts entered the duodenum separately or were separated by a septum which extended to within 2 mm of the common orifice The point is of interest largely because of the commonly held belief that a stone at the common orifice which would close the ampulla would cause bile to enter the pancreatic ducts and possibly cause acute pancreatitis The theory is improbable for several reasons The necessary anatomical relations are relatively infrequent the secretory pressure of bile is insufficient to force a significant amount of bile into the pancreas (5, 6), (see also Chap VIII, Secretory Pressure) and bile when injected into the pancreatic ducts experimentally has failed to cause pancreatitis unless enough force is used to rupture the ducts (Archibald, also Nordman, quoted by Reinhoff and Pickrell (2))*

It seems much more probable that when acute pancreatitis follows occlusion of the common orifice the damage is caused by activation of the pancreatic enzymes in the mixture of bile and pancreatic juice in the ampulla as pointed out by Popper (20) The process of activation may then spread through the juice in the pancreatic ducts by autocatalysis (see under "Trypsin and trypsinogen " Chap III)

The arrangement of the ducts in the dog follows the same general plan as in the human except that the duct of the dorsal rudiment which, in the human, arises between the stomach and the liver bud, in the dog apparently arises caudad to the point of origin of the liver, at least it occupies this position in the adult in which it becomes the major pancreatic duct, the duct

*See also Tejerina-Fotheringham Gastroenterology 10 687 (1948)

associated with the common bile duct becomes the accessory duct The accessory duct is a relatively important channel in the dog since, according to Bottin (7), it is patent and anastomoses with the principal duct in all cases Bottin found a third duct in 20 to 25 per cent of his animals which enters the duodenum midway between the biliary papilla and the opening of the major duct, it communicates with the other ducts but is very small Additional anomalous ducts have been described (8) In the cat the development more nearly follows the human pattern, the pancreatic duct being closely associated with the common bile duct

Continued growth and division of the smaller intralobular branches of the pancreatic ducts produce the definitive secretory portions of the gland, acini and islets of Langerhans

BLOOD SUPPLY

The blood supply of the pancreas has been investigated by numerous workers, the more recent being Pierson (9), Ziegler (10), Kirk (11), and Michels (12) According to the latter, the arteries that supply the pancreas are substantially as follows (references are to Fig 3)

1 Anterior and posterior pancreaticoduodenal arcades about the head of the pancreas The anterior arcade is formed by the anterior superior pancreaticoduodenal (ASPD), a branch of the gastroduodenal The posterior arcade is formed by the textually unlisted retroduodenal (RD), or posterior superior pancreaticoduodenal (PSPD), the upper, nearly invariable, first branch of the gastroduodenal The arcades may end in the superior mesenteric via a common inferior pancreaticoduodenal in which case the anterior pancreaticoduodenal artery sends a dorsal branch to pick up the retroduodenal arcade,

or each arcade may end in the superior mesenteric via its own inferior pancreaticoduodenal artery (IPD) as shown in Fig 3

2 Dorsal pancreatic (DP)* is an unlisted artery of variable size (1-4 mm) distributed to the dorsal surface of the pancreas near the junction of the head and body where the splenic vein joins the superior mesenteric vein to form the portal vein The dorsal pancreatic has a varied origin, from the celiac or aorta, from the first part of the hepatic or splenic, or from the superior mesenteric

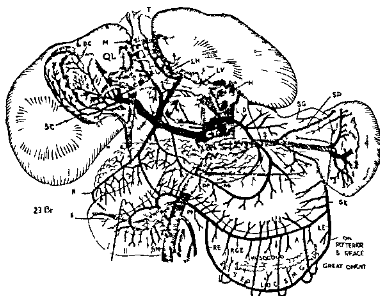


Figure 3

Relations of the pancreas to the regional blood vessels and organs according to Michels based on dissection of 200 →

*Superior pancreatic of Pierson (9)

3 Transverse pancreatic (TP) * This relatively large artery courses along the inferior surface of the pancreas It usually arises as the most prominent left branch of the dorsal pancreatic but may arise separately from the right gastroepiploic or gastroduodenal

4 Splenic branches Two to six small branches which arise from the splenic along its course and which unite with neighboring branches At the distal



bodies With minor variations this arterial pattern was found in 52 per cent of the bodies Duodenum is turned forward to show distribution of the retroduodenal artery (RD) (posterior superior pancreaticoduodenal (PSPD)) and its arcades to the posterior surface of duodenum and head of pancreas It unites with the superior mesenteric (SM) via its own inferior pancreaticoduodenal (IPD) The anterior arcade on the head of the pancreas and anterior surface of the duodenum begins with the (anterior) superior pancreaticoduodenal (ASPD) which unites with the superior mesenteric (SM) via its own inferior pancreaticoduodenal In every instance there are two pancreaticoduodenal arcades one anterior and one posterior

The dorsal pancreatic (DP) here arises from the superior mesenteric (SM) To the left it gives off the transverse pancreatic (TP) which after coursing along the inferior surface of the pancreas anastomoses with the a pancreatica magna (PM) here a branch of the left gastroepiploic (LGE) A right branch joins the right gastroepiploic (RGE) The supraduodenal (SD) of Wilkie supplies the first part of the duodenum with branches of the head of the pancreas

Hepato lieno gastric celiac trunk with hepatic dividing into its three typical branches viz right left and middle hepatic the latter for quadrate lobe Upon dissection 23 hepatic branches were found entering the liver substance the branches being selectively distributed to various intrahepatic areas

*Inferior pancreatic of Pierson (9)

third of the pancreas the splenic gives off a very large branch, the arteria pancreatica magna (PM) It is directed obliquely toward the left, i e toward the tail of the pancreas

5 Branches from the supraduodenal artery of Wilkie (SD) This small artery has a varied origin from the gastroduodenal right or left hepatic right gastric or retroduodenal

6 Branches from the gastroduodenal right gastroepiploic and right gastric to the head and proximal portion of the pancreas (GD, RGE and RG)

7 Branches from the left gastroepiploic (LGE) and splenic terminals to the tail of the pancreas one being especially prominent viz , the arteria caudae pancreatis

Anomalous arterial conditions are often present the most important being a replaced or accessory right hepatic from the superior mesenteric passing through the head of the pancreas or immediately behind it When of the latter variety the right hepatic may receive the retroduodenal arcade via an inferior pancreaticoduodenal or give rise to the dorsal pancreatic with branches to the uncinate process In instances the entire hepatic trunk arises from the superior mesenteric and passes through the head of the pancreas, a fact to be remembered in every resection of this region

INTRAPANCREATIC CIRCULATION

Within the gland the acinous cells and the islets of Langerhans are supplied by separate arterioles Those which supply the islet tissue break up into large, tortuous sinusoids which lie between the islet cells and drain into smaller capillaries which surround the adjacent acini Other acini are supplied with capillaries which arise directly from arterioles (11 13 14) Thus it happens that the acinous cells in

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the vicinity of the islets are exposed to blood which has recently been in contact with islet cells and may contain more of the internal secretion of these cells than the blood supplied to other acini

NERVE SUPPLY

The pancreas receives an abundant nerve supply from both the vagi and splanchnic nerves The function of these nerves will be the subject of a later chapter According to Richins (15), who made his studies on cats by strictly morphological methods, the entire extrinsic nerve supply to the pancreas passes through the celiac plexus and reaches the gland via the nerve plexus surrounding the arteries, chiefly the superior pancreaticoduodenal artery The vagus fibers end in the intrinsic ganglia of the pancreas and from these the parasympathetic path is continued through postganglionic unmyelinated fibers to the acinous cells, islet cells, and the smooth muscle of the ducts The efferent splanchnic fibers destined to supply the pancreas, according to this author, all end in the celiac and associated ganglia and from there the sympathetic path is continued through unmyelinated postganglionic fibers which are distributed solely to the pancreatic blood vessels However, the presence of splanchnic secretory fibers in the cat which do not synapse in the celiac ganglia is indicated by the physiological studies of Babkin Hebb and Sergeyeva (16) and others Myelinated visceral afferent fibers from the pancreas are also present in the splanchnic nerves (15)

Guillaumie (17) using physiological methods on dogs, reached quite a different conclusion regarding the course of the vagus fibers Confirming with few exceptions, the early observations of Popielski (18), she concluded that a majority (80 per cent) of the vagus fibers to the pancreas course along the lesser curvature of the stomach to cross the pylorus and

descend a certain distance in the duodenal wall before entering the pancreas The remaining 20 per cent traverse the hepatic plexus and join the pancreas in the region of the pylorus

These differences in experimental observations may be ascribed to species differences or to differences in the method of study They serve, in any case, to point out the unsatisfactory state of our knowledge of the innervation of the pancreas One important point about which we know nothing concerns the relation of the intrinsic pancreatic ganglia to the ganglionic plexus of the intestine Are the pancreatic ganglia derived from the intestinal plexus? Do they have structural and functional relations with the intestinal ganglia in the adult animal? Are stimuli which are conducted through the intestinal plexus also conducted to the pancreas? Can such stimuli influence pancreatic function? Answers to these questions might serve to illuminate many obscure features of pancreatic physiology

It is often incorrectly stated that the pancreas receives a greater part or all of its vagus nerve supply from the right vagus This error arises from the tendency to overlook the fact that after the vagi merge in the esophageal plexus the right and left nerves can no longer be distinguished Two or more trunks emerge from the esophageal plexus and the trunk which occupies the more posterior position at the level of the diaphragm, although referred to by some anatomists as the right vagus, contains fibers from both the right and left vagi as they occur in the neck When stimulated in the neck the right and left vagi are equally effective in modifying pancreatic function (17) See also Figs 19 and 20, Chap VII

MICROSCOPIC ANATOMY

When studied microscopically the pancreas is seen to be made up chiefly of groups of cells forming

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tains the spherical nucleus This zone exhibits a variable degree of striation produced by an alignment of mitochondria perpendicular to the basal surface of the cell (Fig 4), variation in its appearance is ap-

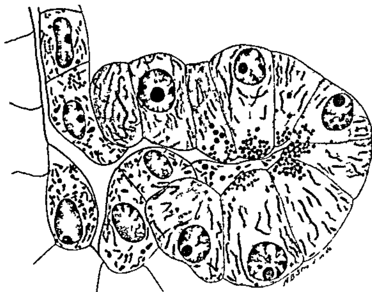


Figure 4

Section of an acinus of the pancreas of the guinea pig showing centroacinous cells with mitochondria and fuchsinophile bodies In the acinous cells may be seen zymo gen granules * and long mitochondrial filaments Acetic osmic bichromate aniline and acid fuchsin methyl green X1555 (From R R Bensley Am J Anat 12 297 1912)

parently related to the functional state of the cell With proper methods of preparation the basal zone shows, also, a very finely granular basic-staining

*But few granules are shown The more usual appearance of the zymogen granules in the resting cell is shown in Figure 22a

acini which tend to be spherical or ovoid in general contour but in the closely packed tissue of the pancreas are actually polygonal Groups of acini form primary lobules, also polygonal in contour, which are imperfectly separated from other primary lobules by incomplete connective tissue septa Numerous adjacent primary lobules form a secondary lobule The secondary lobules are completely surrounded by connective tissue and can be dissected out as separate structures connected with the rest of the gland by ducts, nerve fibers, lymphatic vessels, and blood vessels

The larger ducts (interlobular ducts) run in the connective tissue septa where they give off branches, generally at right angles, which enter the primary lobules to become the intralobular ducts Within the lobule these ducts branch profusely to reach the individual acini where they terminate Within the connective tissue septa also, but not associated with the ducts, are the blood vessels, lymphatic vessels, nerve fibers and intrinsic ganglia of the pancreas Within the lobules, in close contact with the acinous cells lie the islets of Langerhans

The pancreatic tissue proper is composed of acinous cells, islet cells and duct cells The acinous cells, owing to the spheroid shape of the acini, tend to be pyramidal with the truncated apex of the pyramid directed toward the lumen of the acinus They are large cells with a well developed nucleus and nucleolus and abundant granular cytoplasm The granules (zymogen) vary in number and position in the cell depending on the state of activity of the gland but tend to be more abundant in the apical (lumenal) region of the cell When very abundant they may occupy the major portion of the cytoplasm displacing the nucleus to the base of the cell, but there is generally a zone free of zymogen granules near the base The basal cytoplasmic zone of the acinous cell con-

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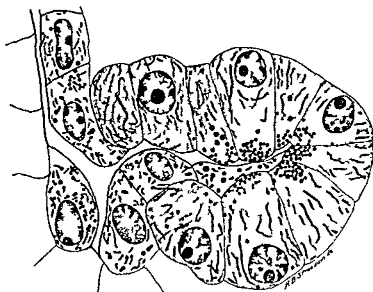


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chromophilic material related to similar material in other zymogenic glandular cells (ex , peptic cells of the stomach, acinous cells of the parotid gland) and, further, is considered by some to be somewhat comparable to Nissl substance of nerve cells Secretory canaliculi have been described in the acinous cells Changes that occur in these cells as a result of secretory activity will be considered in a later chapter

The walls of the larger ducts of the pancreas contain connective tissue consisting of both white and elastic fibers These ducts are lined with columnar epithelium composed of cells with faintly granular cytoplasm The granules react to dyes which stain mucin Ducts of intermediate size are lined with low columnar or cuboidal epithelium and have little or no connective tissue in their walls The small, intralobular ducts are made up of flattened cells with a conspicuous nucleus and relatively little cytoplasm In longitudinal section they appear to be spindle shaped owing to distention of the central portion of the cell by the large nucleus

The manner in which the intralobular ducts terminate in relation to the acini has been difficult to determine Cells corresponding in structure to the cells of the terminal ducts may be seen in microscopic sections within the acini (centroacinous cells, Fig 4) and most authors have concluded that the terminal ducts extend into the lumen of the acinus where they form a sort of lining Zimmermann (19) on the other hand, contended that the "so-called centroacinous cells" do not belong to the acinus in which they appear in microscopic sections but are cells of adjacent ducts which happen to be so situated that they can be seen through the lumen of the acinus His views are not supported by the results of more recent studies

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chromophilic material related to similar material in other zymogenic glandular cells (ex , peptic cells of the stomach, acinous cells of the parotid gland) and, further, is considered by some to be somewhat comparable to Nissl substance of nerve cells Secretory canaliculi have been described in the acinous cells Changes that occur in these cells as a result of secretory activity will be considered in a later chapter

The walls of the larger ducts of the pancreas contain connective tissue consisting of both white and elastic fibers These ducts are lined with columnar epithelium composed of cells with faintly granular cytoplasm The granules react to dyes which stain mucin Ducts of intermediate size are lined with low columnar or cuboidal epithelium and have little or no connective tissue in their walls The small, intralobular ducts are made up of flattened cells with a conspicuous nucleus and relatively little cytoplasm In longitudinal section they appear to be spindle shaped owing to distention of the central portion of the cell by the large nucleus

The manner in which the intralobular ducts terminate in relation to the acini has been difficult to determine Cells corresponding in structure to the cells of the terminal ducts may be seen in microscopic sections within the acini (centroacinous cells, Fig 4) and most authors have concluded that the terminal ducts extend into the lumen of the acinus where they form a sort of lining Zimmermann (19) on the other hand, contended that the "so-called centroacinous cells" do not belong to the acinus in which they appear in microscopic sections but are cells of adjacent ducts which happen to be so situated that they can be seen through the lumen of the acinus His views are not supported by the results of more recent studies

can be obviated to some extent by use of intravenous anesthetics such as chloralose and urethane or one of the barbiturates and by care in exposing and cannulating the ducts A dissection of the ducts in a dog is shown in Fig 5

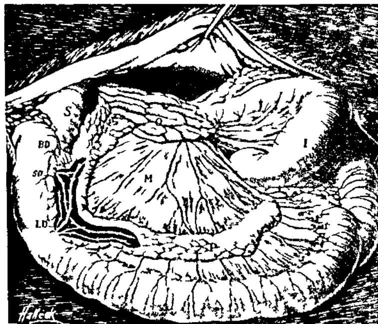


Figure 5

A dissection showing the position and relations of the pancreatic ducts in a dog I intestine O omentum M mesentery S stomach P pancreas (uncinate process) BD position of the bile duct LD large duct SD small duct of the pancreas The pancreas is partly cut away to show the position of the ducts For operations involving the small or accessory duct the duodenum is rotated to the left exposing the posterior surface (From Dennis E Jackson Experimental Pharmacology C V Mosby Co St Louis)

Chapter II

EXPERIMENTAL METHODS

STUDIES of the external pancreatic secretion involve, as a primary necessity, some means of collecting pancreatic juice Any device whereby the pancreatic secretion is diverted to the outside of the body may be described as a pancreatic fistula such fistulas may be either temporary or permanent Permanent fistulas are those that are not incompatible with survival and that persist, after being established, throughout the subsequent life of the animal, all others are classed as temporary fistulas Either type of fistula may be complete or partial depending upon whether all or only a part of the secretion is being diverted

CANNULATING THE PANCREATIC DUCT IN ANESTHETIZED ANIMALS

The simplest temporary fistula is prepared by cannulation of one or both of the pancreatic ducts in an acutely operated animal This method has been used extensively by most students of pancreatic function and continues to yield much valuable information in spite of serious disadvantages There is obvious objection to the use of anesthetic or other depressant drugs in any physiological investigation, in addition the pancreas is extraordinarily sensitive to the inhibitory influences associated with excitement that is apt to accompanny induction of anesthesia and to reflexes associated with the physical trauma of operative procedures These difficulties

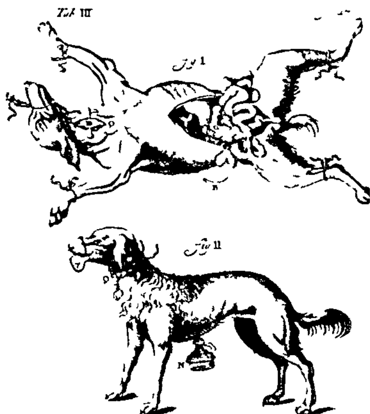


Figure 6

De Graaf s illustration of a temporary pancreatic fistula with collecting vessel the animal pictured in "Fig 2 also has a salivary fistula (From Succi Pancreatici 1671) (Reproduced by J F Fulton Selected Readings in the History of Physiology Charles C Thomas Publisher Springfield Ill)

OTHER TEMPORARY FISTULAS

The first pancreatic fistula was made by Regner de Graaf in the year 1663 and was described the following year in his doctorate thesis (1) One of his illustrations is reproduced in Fig 6 He cannulated the pancreatic duct of a dog with a quill from a wild duck which he selected " because that kind of bird hath longer and thinner than all others " The pancreatic juice was subsequently collected in a small vessel attached to the cannula A similar method was employed by Claude Bernard (2), who used a silver tube, and by Bernstein (3) and others The method was of limited usefulness because the cannula always came out of the duct within a few days

Rous and McMaster (4) in 1923 described their technique for semipermanent intubation of the bile *duct and in 1927 Elman and McCaughan (5) adapted* this method to the pancreas In this procedure (Fig 7, H) the duct is cannulated and the cannula connected to a long, flexible rubber tube, a large part of which is allowed to remain within the peritoneal cavity The free end is drawn out through a stab wound in the abdominal wall and protected by a sterile collecting bag Scott (12b) found this fistula to be unreliable because of early failure of the flow due to clogging of the cannula

PERMANENT PANCREATIC FISTULAS

Among the earliest successful permanent fistulas of the pancreas is that devised by Pavlov (6) (Fig 7, E) *He cut out an oblong portion of the duodenal* wall surrounding the opening of one of the pancreatic ducts (generally the larger) and, after repairing the duodenum, transplanted the excised papilla with the duct attached into the skin of the abdominal wall The operative technique has been described by Babkin (7)

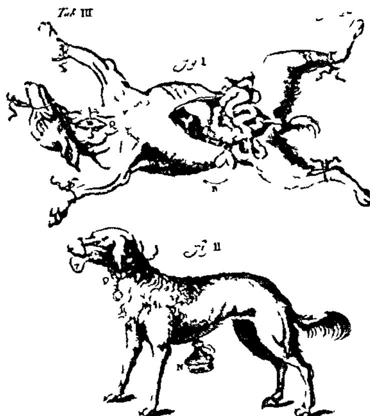


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De Graaf s illustration of a temporary pancreatic fistula with collecting vessel the animal pictured in "Fig 2 also has a salivary fistula (From Succi Pancreatici 1671) (Reproduced by J F Fulton Selected Readings in the History of Physiology Charles C Thomas Publisher Springfield Ill)

and (in English) by Boldyreff (8) A slightly different operation for the same purpose proposed by Heidenhain (9) (Fig 7, B) has no advantage over Pavlov's method Pavlov's operation requires a fair degree of surgical skill and the animals that survive are subject to considerable discomfort owing to the corrosive action of the pancreatic juice in which the proteolytic enzymes are activated by contact with the transplanted mucosa

The objections to the Pavlov fistula were largely obviated by a modification adopted by Babkin in 1904 (see Babkin, 7) After a fistula had been prepared by Pavlov's method and the wound healed he excised the transplanted mucosa completely and sutured the walls of the duct to the margins of the wound Closing of the duct by scar tissue was prevented by daily probing and the juice was collected when desired through a glass cannula inserted into the duct to the depth of about 1 1/2 cm When the cannula was not in place compression of the duct by the scar prevented drainage of the juice to the outside so that most of it flowed into the intestine via the accessory duct If any drained to the outside it was not corrosive since it contained no active trypsin (See Chap III) Animals so prepared lived for three to four years in good health

Direct transplantation of the duct into the skin without the papilla has been proposed from time to time (8, 10) The most successful technique for this purpose is that devised by Inlow (11) (Fig 7, G) He implanted that portion of the intact duodenum containing the pancreatic papilla into the subcutaneous tissue of the abdominal wall At the same, or preferably, a subsequent operation, the intramural portion of the duct was dissected out and cut near the papilla and the free end brought out through the skin near the site of the original incision A catheter was inserted into the duct to facilitate handling and left in

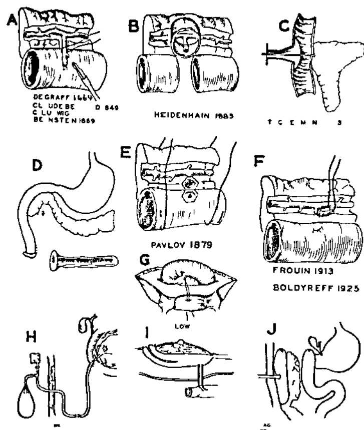


Figure 7

Sketches (diagrammatic) representing various types of external pancreatic fistula used by different investigators (Modified from J M McCaughan (14) Am J Digest Dis)

place until it was expelled spontaneously This fistula has the advantages of Babkin's modification of the Pavlov fistula and is less difficult to prepare Details of the technique are given by the original author and, more fully, by Scott (12a)

The fistula described by Dragstedt et al (13) (Fig 7, J), like that of Pavlov, yields pancreatic juice which contains active trypsin These investigators isolated a portion of the upper duodenum by separating it from the stomach and lower duodenum The pyloric portion of the stomach was excised and the lower duodenum anastomosed to the gastric stump The common bile duct was transplanted into the stomach or lower duodenum The isolated segment of duodenum which included the pancreatic papillae was closed surgically at both ends and a gold-plated metal cannula was inserted through its wall and brought to the outside through the abdominal wall to provide drainage for the mixed pancreatic and duodenal secretions One advantage claimed for this method over the original Pavlov fistula is that the juice, draining through a cannula, does not spread over the skin and cause irritation and erosion An obvious disadvantage is that the extensive surgery in the vicinity of the pylorus is almost certain to result in severing an important part of the nerve supply of the pancreas (15) A modification of this fistula suggested by the original authors together with an admirable description of the surgical technique is given by Scott (12a)

An ingenious and original method of preparing a permanent fistula which yields pure pancreatic juice has recently been described by McCaughan (14) (Fig 8) His method is based on the demonstrated facts that the pancreatic ducts anastomose freely within the gland and the juice may be made to flow in either direction Both pancreatic ducts in a dog were exposed and cut between ligatures The uncinate process was freed from its mesenteric attachments and its lower end brought out through a wound in the abdominal wall and secured to the wall by sutures The exposed end was then amputated and after healing, the juice was collected through a cannula from

the severed duct of the uncinate process McCaughan reports no dilatation of the ducts when these were examined at autopsy and no atrophy of acinous tissue

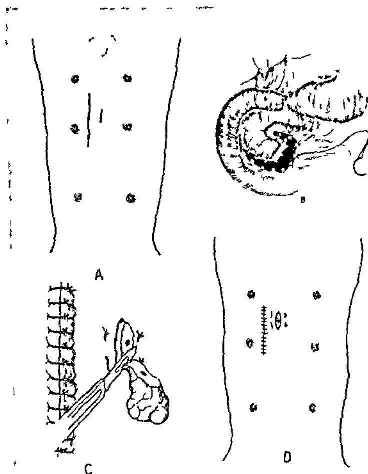


Figure 8

Technique of the retrograde external pancreatic fistula as illustrated by McCaughan (From J M McCaughan (14) Am J Digest Dis)

such as might be expected to occur if the drainage were not adequate and complete The author credits Senn (16) with a somewhat similar technique but Senn's fistula drained only a portion of the pancreas and resulted in ultimate failure of secretion owing to atrophy of the gland

No pancreatic fistula yet devised is entirely satisfactory Complete fistulas such as those of *Elman and McCaughan* (5), *McCaughan* (14) and Dragstedt's fistula as originally described (17) always result in death of the animal unless the fluid and base lost through the pancreas is regularly replaced, the survival time is short in any case Furthermore they cause disturbances of digestion and absorption which cannot be wholly corrected by any means yet devised Partial fistulas such as those of Pavlov (6), Babkin (7), Inlow (11) and others yield an uncertain fraction of the pancreatic juice and are, *therefore*, quantitatively unsatisfactory

V Brown Scott (12b) who has prepared and studied more than 200 pancreatic fistulas finds the mortality excessively high in animals with the Pavlov type fistula This difficulty has also been emphasized by Babkin (7) Animals with the original Dragstedt fistula survived only a short time in Scott's experience even when given electrolytes intravenously He recommends the Inlow type fistula for beginners because it is relatively easy to prepare but warns that the survival time of the animal will be inversely related to the volume of pancreatic juice lost through the major duct He found that even with this type of fistula the pancreatic juice had a corrosive action on the skin He emphasizes, as do all who have had experience *with pancreatic fistulas, the tendency to hypersecretion* in fistula animals Hypersecretion is a major cause of death and, during the period of survival, it may give rise to abnormal or misleading experimental results (see Chap IV under Pancreatic fistula)

OTHER METHODS

Pancreatic juice can however, be collected at will, quantitatively and in a pure state without a permanent pancreatic fistula L B Tuckerman, an American surgeon, (17) who seems to have been the first to demonstrate this fact prepared a fistula of the duodenum in a dog and fitted it with a metal tube with its inner end opposite the opening of the pancreatic duct Through this fistula he was able to insert a glass cannula into the duct and collect the juice This method proved impractical (12b, 14) for the study of pancreatic function until Thomas and Crider (18) prepared a duodenal fistula using a more conveniently shaped fistula tube with a larger bore than that used by Tuckerman In their first experiments they collected the juice through a soft rubber funnel pressed against the mucosa surrounding the papilla (Fig 9) Pancreatic secretion collected in this manner is similar to that obtained with the Pavlov fistula in that it is mixed with a small amount of duodenal secretion and therefore contains active trypsin Scott and his co-workers (19) modified the procedure by cannulating the pancreatic duct through the duodenal fistula and obtained pure pancreatic juice in which the trypsin was inactive By ligating the accessory duct while preparing the duodenal fistula all the pancreatic juice can be made to flow through the main duct and subsequently collected quantitatively through a cannula (see Fig 10)

One advantage of this method is that there is no loss of pancreatic juice in the intervals between experiments and therefore digestion and acid-base balance remain normal A most important advantage is that extensive surgical procedures involving the pancreas and associated organs are avoided, consequently the normal nerve supply and blood supply are retained This is undoubtedly the method of choice

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vantage is that the animal must be under continuous observation while the juice is being collected This difficulty can probably be overcome but until it is, the method is not applicable to problems requiring collection of the secretion over long periods of time

Numerous other methods applicable to special problems have been used but cannot be described in detail here Farrell and Ivy (20) transplanted a portion of the pancreas into the mammary gland of a lactating bitch and studied the secretion after severing the original blood and nerve supply Houssay and Molinelli (21) transplanted the pancreas and duodenum into the neck in acute experiments by anastomosing the blood vessels Babkin and Starling (22) and others

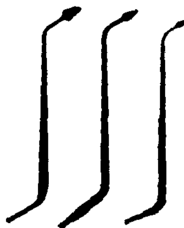


Figure 10

Cannulas used to cannulate the pancreatic duct through a duodenal fistula after the method of Scott Cannulas are of pyrex glass They were filled with a contrasting paste before being photographed (From Hart and Thomas (24) Gastroenterology)

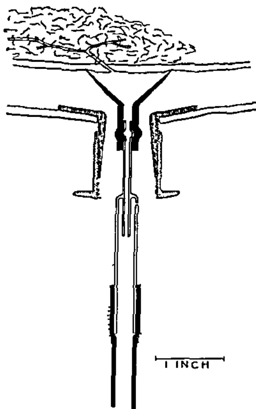


Figure 9

Diagram of duodenal fistula opposite the main pancreatic papilla illustrating the method of collecting juice originally used by Crider and Thomas The same type of duodenal fistula was used by Scott and later by Hart and Thomas (24) in cannulating the duct (From Thomas and Crider (18) Am J Physiol)

for most types of experimental work on the pancreas The duodenal fistulas are easily made by any amateur surgeon and the technique of cannulating the duct is readily learned The animals require no special care and remain in good health indefinitely A disad-

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(23) have described methods for perfusing the excised pancreas

The study of pancreatic function in human subjects will be discussed in a subsequent chapter

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the pH of more than 60 specimens of pancreatic juice, collected from healthy dogs by the method of Scott (Ref 19, Chap II) The samples exhibited considerable differences in their general properties, nevertheless except for a very few specimens the pH values remained within a fairly narrow range, namely pH 8 0 to pH 8 3 The authors expressed the opinion that the extremely high or low pH values occasionally reported were either in error or abnormal and that pancreatic juice, at least in the dog, is secreted at a fairly constant pH

Specific gravity The specific gravity of dog's pancreatic juice as measured gravimetrically at room temperature ranges from 1 007 to 1 042 depending on the amount of protein in the juice Values as low as 1 005 have been reported for human juice but these are probably in error or obtained on abnormal secretion since 1 007 is approximately the specific gravity of a protein-free bicarbonate solution corresponding in concentration to pancreatic juice

In a study of more than 200 samples of pancreatic juice obtained from dogs Crider and Thomas (9) found a linear relation between specific gravity and total nitrogen (Fig 11) Specific gravity was estimated by weighing the juice in a 1 ml specific gravity bottle at room temperature and the nitrogen was measured by the micro-Kjeldahl method The relation between specific gravity and total nitrogen is represented by the following equation

$$\text{Total N (mg /cc)} = 594\ 8\ (\text{Sp g} - 1\ 0075)$$

Osmotic activity The osmotic activity of pancreatic juice is the same as that of the blood when the two are collected simultaneously from the same animal When the osmotic activity of the blood is altered experimentally the pancreatic juice shows a corresponding change (10, 11)

Chapter III

PANCREATIC JUICE

THE amount of pancreatic juice secreted varies with the individual and the diet, hence only rough approximations of the usual 24 hour volume are possible Babkin (1) gives an estimate of 17 to 22 cc per kilo per day for dogs and the results of recent work (2, 3) are reasonably consistent with this figure Corresponding data on human subjects (1, 4, 5) indicate a somewhat wider range but about the same average Because the intestinal contents are generally acid in reaction (6) it may be assumed that the 24 hour volume of pancreatic juice is less than the amount of gastric juice secreted in the same time The latter has been estimated (7) at about 25 cc per kilo per day

1 *PROPERTIES OF PANCREATIC JUICE*

Physical Properties Pancreatic juice is generally a colorless, odorless, alkaline fluid of low viscosity, tasting strongly of sodium bicarbonate Some specimens have been described as having a faint straw color Exceptional specimens, having a high concentration of enzymes, may be viscous and may even jell at low temperatures

pH The literature contains estimates of the pH of pancreatic juice, too numerous to cite, ranging from pH 7 1 to pH 9 0, but many of these are of little value owing either to inadequate methods of estimation or to the juice having been collected under abnormal conditions Hart and Thomas (8) measured

the pH of more than 60 specimens of pancreatic juice, collected from healthy dogs by the method of Scott (Ref 19, Chap II) The samples exhibited considerable differences in their general properties, nevertheless except for a very few specimens the pH values remained within a fairly narrow range, namely pH 8 0 to pH 8 3 The authors expressed the opinion that the extremely high or low pH values occasionally reported were either in error or abnormal and that pancreatic juice, at least in the dog, is secreted at a fairly constant pH

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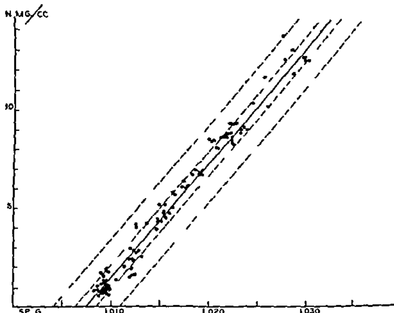


Figure 11

Graph showing relation between specific gravity and total nitrogen in 100 samples of dog's pancreatic juice Data for an additional 100 samples obtained from a different group of dogs fell within the same range

2 COMPOSITION OF PANCREATIC JUICE

Inorganic constituents The distinguishing chemical characteristic of pancreatic juice is its high bicarbonate content The fact is now well established (8, 11, 12, 13, 14) that the bicarbonate and chloride concentrations vary in a reciprocal manner so that the sum of the two expressed in milliequivalents is constant and nearly the same as the total base of the blood plasma, also that within limits the bicarbonate concentration increases and the chloride decreases with increasing rates of secretion Hart and Thomas (8) found that the direct relation between bicarbonate

concentration and rate of secretion was limited to rates below 0 05 ml of juice per minute per kilo of body weight At higher rates the bicarbonate concentration attained a constant maximum characteristic of the individual animal (Fig 12) Maxima ranged

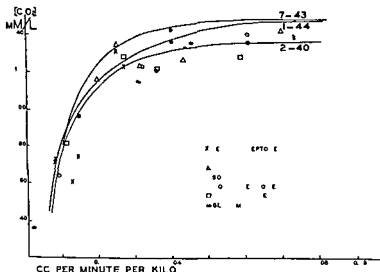


Figure 12

Curves showing the relation of bicarbonate concentration (measured as total CO_2) to the rate of secretion of pancreatic juice from three dogs Curves were drawn by inspection Points are shown for only one curve that for dog 2 40 The various symbols indicate the type of stimu lus used to elicit secretion (From Hart and Thomas Gastroenterology 4 417 1945)

between 135 and 148 milliequivalents per liter, minimal concentrations approximated that in blood plasma In addition to chloride and bicarbonate, the pancreatic juice contains a small amount of phosphate (11) but less than occurs in blood plasma

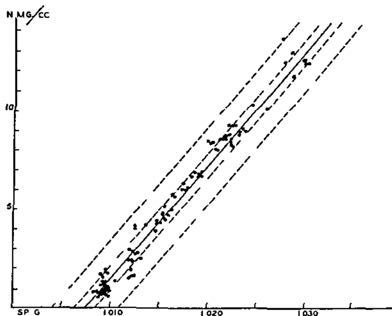


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pending on the dog under observation As a rule an additional boundary appeared indicating the presence of still another component, if the buffer used was sodium diethylbarbiturate at pH 8 6 The mobilities of the various fractions in the electric field corresponded closely to those of various globulin fractions of blood plasma but no fraction was found with the higher mobility characteristic of albumen The relative concentrations of the several fractions were fairly constant regardless of the concentration of total protein These observations encourage the speculation that the four constant fractions separable by electrophoresis correspond to the several enzymes known to be present in pancreatic juice The inconstant fifth fraction may have been mucin Should these conjectures prove to be correct the data would support the view that the several pancreatic enzymes exist in the juice as individual proteins, also that they are secreted in parallel concentration

3 ENZYMES OF PANCREATIC JUICE

Pancreatic juice promotes hydrolysis of all three classes of foodstuffs and is in fact the most active and versatile of all the digestive secretions It owes its activity to the presence of proteolytic, amylolytic and lipolytic enzymes and to the inorganic constituents which provide a favorable medium for their activity

Trypsin and trypsinogen The proteolytic activity of pancreatic juice is due to the presence of two or more enzymes, the best known of which is trypsin The term trypsin was used by Kühne (23) to designate the proteolytic agent which he found to be present in certain samples of pancreatic juice He noted that fresh pancreatic juice and extracts of fresh pancreas were devoid of proteolytic activity This fact was further elucidated by Heidenhain (24) who concluded

The principal bases of pancreatic juice are sodium, potassium, and calcium The concentrations of sodium and potassium are equivalent to those of the blood plasma but the calcium concentration is only 3 to 4 mg per cent (14, 15) The low calcium concentration compared to that of the blood (10 mg per cent) is attributed by Komarov et al (14) to the fact that much of the blood calcium is combined with protein and is not in a diffusible state It is certainly not due to impermeability of the pancreatic cells to calcium since Ågren (16) has shown that calcium may be excreted by way of the pancreas

Protein The protein content of pancreatic juice varies in a complicated but orderly manner with the conditions governing secretion A rough calculation of the protein concentration in 200 samples of dog's pancreatic juice studied by Crider and Thomas (9) based on the total nitrogen minus an assumed 20 mg per cent of non-protein nitrogen (14) in all samples indicates an extreme variation between 0 1 per cent and 10 0 per cent Concentrations found in human pancreatic juice collected through accidental or surgical fistulas (15, 17, 18, 19) are nearer the lower extreme, ranging from 0 1 to 0 3 per cent It is probably true that human pancreatic juice consistently contains less protein than that of the dog but the difference may be less than these data suggest since during the hypersecretion that often complicates chronic pancreatic fistulas the protein content is always low

Chemical fractionation of human pancreatic juice by Glaessner (17), Ellinger and Cohn (18) and Wolgemuth (19) indicated the presence of albumen and globulin Munro and Thomas (20) subjected pancreatic juice of dogs to electrophoretic analysis in the apparatus described by Tiselius (21) They found that in sodium bicarbonate buffer at pH 8 2 the protein of the juice separated into four or five components de-

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that the cells of the pancreas contain no proleolytic ferment but a precursor which he called a 'zymogen" More recently Northrop (25) and Kunitz (22, 26) working together have isolated in crystalline form two proteolytic enzymes and their precursors (zymogens) from pancreatic extracts They named the enzymes trypsin and chymo-trypsin respectively It should be noted that the term trypsin has long been used in a broad sense to designate the total proleolytic activity of pancreatic juice and the term trypsinogen to refer to the inactive precursors of the active material In this discussion these terms are given the more limited meaning assigned to them by Kunitz and Northrop

Trypsin is a protein (mol wt 34,000) which may be prepared from active pancreatic juice, pancreatic tissue extracts, or from trypsinogen by exposing it to specific activating agents As an enzyme it hydrolyzes native protein producing peptones and peptides It accelerates coagulation of blood, in which it acts as a thrombokinase, but has only a feeble action in clotting milk Its optimum pH for digestion of casein lies between pH 8 0 and pH 9 0 When pure it is reversibly inactivated by heat in acid solution it slowly decomposes (auto-digestion?) in alkaline solution

Trypsinogen is likewise a protein, indistinguishable on chemical analysis from trypsin, but has different solubilities and a different crystalline form It exhibits no proteolytic activity Trypsinogen has been prepared from extracts of fresh pancreatic tissue and it undoubtedly occurs in proteolytically inactive pancreatic juice as the precursor of trypsin Trypsinogen in solution changes spontaneously to active trypsin but the change is accelerated by acid or by exposure to concentrated $MgSO_4$, $CaCl_2$, active trypsin, or to one of a group of enzymes called kinases The effectiveness of trypsin in the activation of trypsinogen is of great importance because

such autocatalytic reactions progress with great rapidity, once they are started, and require only minute amounts of activating agent to set them going Spontaneous activation is suppressed in crude extracts and doubtless also in the living pancreas by the presence of an inhibitor (22) which inactivates small amounts of trypsin

Enterokinase Pancreatic juice as secreted in the living animal contains trypsinogen rather than active trypsin The trypsinogen is activated on contact with the intestinal mucosa by means of a special activating agent called enterokinase (28) The chemical nature, origin, and mechanism of formation of this substance are obscure It does not appear in the intestinal secretion in the absence of pancreatic juice (48b, 29) but is present in intestinal mucosal extracts A similar substance is present in pancreatic extracts (30) Although long subject to controversy, the kinases are now believed to be enzymes (27) as Schepowalnikow (28) originally assumed It has been reported, but not confirmed, that enterokinase disappears from the intestinal mucosa after pancreatectomy (29) Kunitz (31) has observed that certain common molds (penicillium) produce an enzyme capable of activating trypsinogen

Chymo-trypsin and chymo-trypsinogen Chymotrypsinogen is a protein substance (Mol wt 40 000) which occurs in pancreatic extracts and, presumably, also in pancreatic juice It is converted to chymotrypsin only by active trypsin but indirect activation can be accomplished by enterokinase if trypsinogen is also present to form trypsin Chymo-trypsin is a proteolytic enzyme with activity comparable to that of trypsin but having different specificities, for example, it coagulates milk but not blood Chymotrypsin digests casein somewhat more rapidly than does trypsin but the digestion is carried much farther by a mixture of the two enzymes than by either

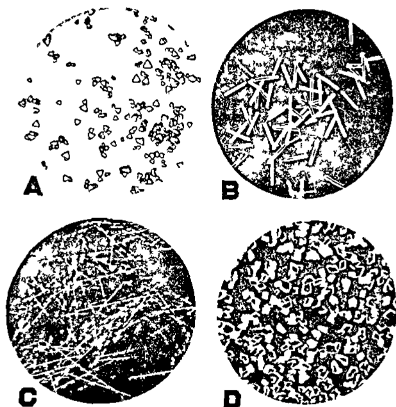


Figure 13

Crystalline enzymes and their precursors A trypsino gen B trypsin C chymo-trypsinogen D chymo trypsin (From Northrop Crystalline Enzymes Columbia Univ Press, New York)

alone, a fact which indicates that the two enzymes attack different linkages in the protein molecule Like trypsin, chymo-trypsin is reversibly inactivated by boiling in acid solution Its optimum activity occurs in the same pH range as that of trypsin (pH 8 0 to pH 9 0 for casein digestion) (27)

Peptidases The peptides resulting from the digestion of native protein by the combined action of pepsin, trypsin and chymo-trypsin and, possibly, other enzymes as yet unknown are broken down to their constituent amino acids by a group of enzymes known as peptidases (the erepsin of the older literature) These enzymes occur principally in the intestinal mucosa but some have been found in pancreatic extracts The only one so far isolated is carboxypeptidase, an enzyme which hydrolyzes peptides containing a free carboxyl group but no free amino group One may infer from the fact that this enzyme is present in the pancreas in sufficient abundance to permit of its being obtained in crystalline form (Anson, 32) that it is probably secreted in the juice as a digestive enzyme In fresh pancreas it occurs in an inactive form, procarboxypeptidase, and, like chymo-trypsinogen it is activated by trypsin

Pancreatic lipase The ability of pancreatic juice to emulsify and hydrolyze fats is probably due to a single enzyme (33, 34), pancreatic lipase This enzyme is known principally through its activity and has not been isolated in a pure state or crystallized except possibly once as an accidental observation (35) A prolipase which is activated by serum has been described as occuring in pancreatic extracts but it is not clear that the lipase secreted in the juice requires such activation The lipase of pancreatic juice hydrolyzes fat, only in the presence of bile salts or other substances with similar properties, for example, some of the synthetic detergents* (36) Bile salts in high concentration inhibit the action of lipase as do salts of certain heavy metals (Cu Fe, Co) and halogens (F I Br) The pH at which pancreatic lipase exhibits its optimum activity varies

*Polyoxyalkylene derivatives of sorbitan monolaurate such as Tween "

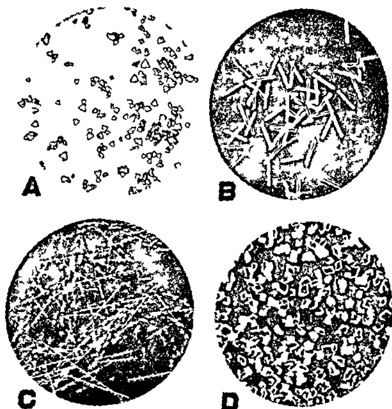


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(2) Chronic differences in the relative concentrations of the different enzymes related to the composition of the habitual diet

(3) An acute increase in the output of a single enzyme when its substrate is used as a stimulus for secretion

It is now generally understood that the total enzyme concentration of pancreatic juice is determined to a great extent by the type of stimulus used to provoke secretion This mode of adaptation arises from the fact that there are two classes of stimuli for the pancreas, one that promotes secretion of enzymes chiefly and another that is mainly concerned with the secretion of alkaline fluid These classes of stimuli and their effects on the composition of pancreatic juice will be considered at length in subsequent chapters

There is good evidence that the secretion of enzymes by the pancreas is influenced by diet but it is by no means certain that in every instance this represents a specific adaptation in the sense that an increase or decrease in intake of one type of food over a period of time leads to a corresponding change in output of the appropriate enzyme Since secretion of enzymes is an end result of synthesis of new protein by the secreting cells, it is evident that it may be adversely affected or modified by a diet that is inadequate in any essential component

Abramson (41) found that the people in a certain province which he investigated whose diet was practically devoid of meat, fish or eggs appeared to secrete less of all the pancreatic enzymes than the general population He studied the enzymes of the fasting duodenal contents before and after stimulation of the pancreas by means of ether in the intestine The differences from the general population were not statistically significant but he considered them suggestive He quotes Michelson (42) to the effect that

with the substrate but is always on the alkaline side of neutrality

Pancreatic amylase The amylase of pancreatic juice has been isolated as a pure crystalline protein (37, 38) Waldschmidt-Leitz and Reichel (39) claimed to have prepared an active amylase from the pancreas which gave none of the common protein reactions They are now believed to have been misled by the extraordinary potency of this enzyme which is still active in dilutions of 1/100,000,000 Solutions must be about 1,000 times more concentrated than this to give typical reactions for protein Pure amylase will digest 20,000 times its weight of starch in 30 minutes or 4,000,000 times its weight if given sufficient time The enzyme digests glycogen as well as starch, under favorable conditions the end product in both instances is maltose

Pancreatic amylase is secreted in active form but becomes inactive if dialyzed against distilled water owing to removal of neutral salts which are thus proved to be essential for the activity of the enzyme The anions are more important than the cations, the most efficient activator being chloride The optimum pH for the activity of pancreatic amylase varies between pH 6 5 and pH 7 2 depending on the kind and concentration of neutral salt present in the solution

The pancreatic juice is said also to contain maltase and sucrase (40) and under ertain conditions, lactase (43)

Variations in composition Alaptation Alterations in the composition of pancreatic juice brought about by differences in the diet or in the immediate stimuli provoking secretion presen a complex problem which is even now far from a fial solution The following adaptations have been sugested

(1) Differences in the total enzyme concentration or in the bicarbonate content depeding on the stimulus used to evoke secretion

was a significant increase in lipase on both the meat and the fat diets The trypsin was increased on the meat diet and the amylase decreased on the fat diet but these differences were not statistically significant, however, they are in agreement with significant differences found by Grossman and co-workers in their rats on corresponding diets

These data are suggestive but confusing, the behavior of the lipase in animals on a meat diet in particular fails to conform to the requirements of adaptation Atrophy of the pancreas in one series of rats suggests that some of the other changes observed may have been due to an inadequate supply of nutrients necessary for synthesis of enzymes Increase in lipase along with trypsin on the high protein diet also suggests a nutritive factor

The question whether or not the pancreas can increase its output of a particular enzyme when the appropriate substrate is acting as a stimulus to secretion arose as a result of the early experiments of Walther (46) in Pavlov s laboratory which seemed to show such an adaptation Walther s experiments were done before the discovery of enterokinase and the activating effect of bile on pancreatic lipase In consequence the trypsin and lipase in the juice that he studied were only partially active and his enzyme determinations were correspondingly inaccurate and misleading Relying on these results, Pavlov formulated and published his well known theory of the adaptability of the pancreas to the type of food undergoing digestion In 1904 Babkin (47, 1 - p 488) repeated the studies of Walther with appropriate modifications and found that although the total enzyme content of the juice varied with the type of food undergoing digestion the different enzymes were secreted parallel to one another Although the error was thus promptly corrected in Pavlov s laboratory (47, 48a) as soon as the necessary information became

in 10 normal subjects placed on special diets for from 10 to 14 days there was evidence of adaptation to diet in the trypsin and lipase content of the pancreatic juice but none in the amylase

Bainbridge (43) has reported that lactase is present in the pancreas of milk-fed puppies but is absent from the newborn and the adult It reappears in the pancreas of adult dogs if they are put on a milk diet for several weeks He found that it could also be made to appear in the pancreas of adult dogs by means of subcutaneous injections of intestinal mucosa obtained from milk-fed animals It has also been reported (44) that about twice as much lipase can be extracted from the pancreas of fattened hogs as from lean animals

Grossman, Greengard and Ivy (45a) have studied the enzyme content of the pancreas in a series of rats that were fed for several weeks on various diets They found a marked predominance of amylase in the pancreas of animals fed a high carbohydrate diet the trypsin content was low but the lipase values were unchanged Animals fed a high protein diet showed an increase in trypsin and lipase On a high fat diet the trypsin and lipase values were unaltered but the amylase was low There was a decrease in all enzymes and atrophy of the pancreas in animals on a high fat, low protein (10 per cent) diet In a subsequent study (45b) they found that dextrose in the diet increased the amylase content of the pancreas more than starch but that substituting protein hydrolysate for casein depressed the trypsin production

Unpublished experiments carried out by Dr Beamer in our laboratory, using dogs instead of rats and pancreatic juice instead of pancreatic tissue have given somewhat similar results Dogs were fed diets consisting of Purina dog checkers, lean meat or fat Compared with the results on checkers there

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available, the correction never quite overtook the error and "Pavlov's theory" continues to be quoted by the unwary

Subsequent work on animals (48a, 49) and man (50) has generally supported Babkin's conclusions but occasional exceptions have been noted (51, 52, 53) mostly in clinical studies in which fresh, pure pancreatic juice is seldom available The conclusion seems warranted that in studies made over a short period of time in the same individual the enzymes are generally secreted in parallel concentration *

Among the observations that do not fully support this conclusion some studies by Lagerlöf (50) and Christiansen (54) (quoted by Lagerlöf) seem particularly to merit consideration Christiansen reported that in experiments on man after introduction into the duodenum of certain digestive products an increase in the corresponding enzyme may occur Lagerlöf found that the enzymes were secreted in parallel concentration in response to secretin and other artificial stimuli but reported that with alimentary stimuli he was able to confirm Christiansen's observations "in a different way" Although these are "clinical" studies they cannot be disregarded on that account alone since Lagerlöf's methods were good enough to demonstrate parallel secretion of enzymes under other experimental conditions

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*This statement and the supporting evidence are subject to the reservation that direct methods for measuring concentration of enzymes are not yet available In a strict quantitative sense we cannot speak of parallel secretion until we are able to determine the individual enzymes themselves and not their activity as has been done exclusively so far up to date

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Chapter IV

THE FUNCTIONS OF THE EXTERNAL SECRETION

DIGESTION AND ABSORPTION

THE most important function of the external pancreatic secretion is to aid in digestion and absorption of the major foodstuffs Efforts to evaluate this function have been made by observing the results of excluding pancreatic juice from the intestine either by ligating the ducts or removing the pancreas Claude Bernard (1) was apparently the first to give a clear demonstration of the importance of the pancreas for digestion and absorption of fat although the presence of fat in the stools (stearrhea or steatorrhea) had previously been reported as a sign of pancreatic disease (2)

Bernard's findings were frequently questioned by subsequent workers (for literature see Handelsman 3, or Greenberg, 4) Although Abelman (5) in 1890 working with von Mering and Minkowski (6) showed that large amounts of fat and nitrogen regularly appeared in the stools following pancreatectomy, negative results following ligation of the ducts continued to be reported Abelman's results were attributed by some to loss of an internal secretion (Lombroso, 7) Abelman himself suggested that after ligation of the ducts pancreatic enzymes were absorbed into the blood and were then secreted by other glands

After Hess (8) demonstrated the frequent occurrence of anomalous ducts and Pratt, Lamson and Marks (9) pointed out that ligation of the ducts was frequently followed by formation of sinuses which reestablished communication between the pancreas and the intestine, it became evident that many observers had failed in their efforts to exclude all the pancreatic juice from the intestine as Claude Bernard had suggested years before Complete exclusion is essential in such experiments because only a small fraction (one-fifth to one-tenth) of the pancreas is necessary to maintain normal digestion and absorption (15) Modern investigators (4, 10, 11, 12, 13, 14, 15, 16, 26, 36) have generally found a pronounced deficiency in digestion and absorption following either ligation of the ducts or pancreatectomy

TABLE I

R sults btai ed by ri s workers regarding the p t ge f di t ry it ge and fat bso bed i d gs whe th e t rnal ec eti f th panc as was unq stionably cluded from th i testi (Hand l man 1938)

A th s	% Nitrog Absorbed	% Fat Ab bed
P tt (1907) du ts ligated	22 2 61 7	4 8 76 6
Vins tini (1914) duct ligated		28 7 44 0
Vins tini (1914) aft pan tectomy		8 7 25 7
Crui kshank (1915) o tag panc e tectomy	78	32 6
Cruickshank (1915) two stag pan t t my	79 6	72 12
Brugsch (1919 d t ligated	21 8 93 5	0 21 8
Li ht and W gn (1927) du t ligated and afte pan t tomy	55	0
Pratt Fal L and H sch nson (1931) d ct ligated	85 3	93 6
P tt Fal L and He ch nso aft pan t tomy	56 2	95 2
P att (1934) with H nd l man and Gold n du t ligated	47 60	41 5 93 7
S ll (1937) aft pan tectomy		89 51 (ge)
G enbe g (1933) i t du t ligated	51 2 66 8	0 28

F r f nc Hand l man (3)

Results reported by various authors prior to 1938 are shown in the above table (Table I) taken from the review by Handelsman (3)

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The two functions cannot really be distinguished because although neutral fat may be absorbed unchanged, partly digested fat aids in emulsification which is a necessary condition for absorption Changes in motility of the stomach and intestine and in acidity of the intestinal contents, to be described presently, may also contribute to the poor absorption of the various foodstuffs

ABSORPTION OF VITAMINS

Associated with inadequate absorption of fat a corresponding deficit in absorption of the fat-soluble vitamins is to be expected May and McCreary (24) and Pratt and Fahey (25) have shown that in pancreatic fibrosis and in celiac disease the vitamin A content of the blood is less than normal and does not increase as much as it does in normal subjects when the vitamin is given by mouth

THE NEUTRALIZING FUNCTION OF THE PANCREATIC JUICE

The bicarbonate of the pancreatic juice helps to neutralize the acid of the gastric juice as the latter enters the intestine and in this way tends to prevent development of excess acidity in the intestine It aids also in controlling the acidity of the gastric contents through regurgitation of intestinal contents into the stomach The alkaline secretions of the intestinal glands and to a less extent, the bile share in these functions

Hoerner (26) studied the effect on the pH of the duodenal contents of excluding pancreatic juice from the intestine by avulsion of the pancreatic ducts About the same time Loewy (27) reported a similar study in which he ligated the ducts Both found a wide variation in acidity of the duodenal contents before

The variable results obtained in experimental animals as indicated by the data in the table occur also in human subjects For example, of three patients studied by Brunschwig and Allen (14) after removal of the head of the pancreas two absorbed only 8 8 per cent and 4 0 per cent respectively of ingested fat while the third absorbed 91 6 per cent A patient with a similar operation studied by Lake, Cornell and Harrison (13) absorbed 54 per cent of the nitrogen and 71 per cent of the fat intake There is also considerable variation in absorption by individual experimental animals at different times, these differences are not caused by differences in the diet (10) and no other satisfactory explanation has been offered Apparently the general well-being of the subject is a factor (17) and it has been suggested that vitamins play a part (18)

Digestion and absorption of carbohydrate food is also impaired in the absence of pancreatic enzymes, at least in dogs (19, 20, 21), but the deficit is less pronounced and less consistently present than that of the other foodstuffs Various explanations for the relatively good absorption of carbohydrates have been offered (22, 23) but none is entirely satisfactory Doubtless the extraordinary potency of the amylases (Chapter III) is a contributing factor

Deficient absorption of carbohydrate and protein is probably secondary to inadequate digestion since unchanged starch (21) and meat (14) appear in the stools Diminished fat absorption is less satisfactorily explained on this basis, for example, Greenberg (4) found that less than one-third of the fat excreted in the feces as a result of ligating the pancreatic ducts appeared as neutral fat and the greater part consisted of soaps and fatty acids Similar results were reported by Abelman (5) and by Pratt, Lamson and Marks (9) Evidently pancreatic lipase aids directly in absorption as well as in digestion of fat

one factor in the complex etiology of these experimental ulcers

INFLUENCE OF PANCREATIC JUICE ON GASTRIC FUNCTION

As mentioned in the preceding section the neutralizing function of the pancreatic juice may at times be evident in the gastric contents Absence of pancreatic juice from the intestine affects other gastric functions in a way which is not so readily explained An increase in volume and acidity of the gastric secretion following ligation of the pancreatic ducts was observed in dogs with Pavlov pouches by Fauley and Ivy (34), particularly noticeable during the third and fourth hours following a meal, that is during the intestinal phase of gastric secretion Increased acidity of the gastric contents was noted by Hoerner (26) only after ulcers had developed

A decrease in gastric emptying time occurred in Yesko s (29) animals after avulsion of the pancreatic ducts Fauley and Ivy (34) and Nothmann and Wendt (35) observed the same phenomenon after pancreatectomy The former attributed the change to excessive hunger Nothmann and Wendt reported a particularly striking increase in the rate of emptying of fat which normally remains in the stomach longer than any other food After pancreatectomy olive oil or oleic acid left the stomach in less than one hour The progress of such material through the small intestine was also abnormally rapid Nothmann and Wendt were unable to reproduce these changes by simple ligation of the ducts, an observation that lends support to Ivy's theory that the hypermotility is related to the excessive hunger of the depancreatized animal As mentioned previously acid, in contrast to fat, may leave the stomach more slowly in the absence of pancreatic juice

as well as after exclusion of the pancreatic juice but the acidity was, in the averages, increased after operation by about one pH unit Although this may seem a small variation, it is by no means negligible since it represents about a ten-fold increase in hydrogen ion concentration Unpublished experiments of Pratt, Golden and Handelsman mentioned by Handelsman (3) have given comparable results, as have recent experiments by Pincus, Thomas and their associates (28)

Yesko (29) found the acidity of the gastric contents slightly increased after excluding pancreatic juice from the intestine by avulsion of the ducts and Serdjukov (51) and more recently Elman (31) noted delayed emptying of acid from the stomach when the pancreatic juice was drained away through a fistula The latter observation suggests that gastric motility may be inhibited when the intestinal contents are not adequately neutralized

Failure or deficiency of the neutralizing function of the pancreas may, conceivably, be an etiologic factor in peptic ulceration of the duodenum but its relative importance has not been finally determined Comfort and Osterberg (30) were unable to demonstrate any deficiency in the bicarbonate secretion of the pancreas in ten patients having duodenal ulcer Ulceration of the duodenum is a common result of excluding pancreatic juice from the intestine in experimental animals, however, ulcers are much less frequent following ligation of the ducts (15) than when the pancreatic juice is drained away through a fistula (32) or into the lower ileum (33) According to Dragstedt (15) pancreatectomy in dogs only rarely causes duodenal ulceration It seems obvious that some other mechanism than failure of the neutralizing function of the pancreas is involved in production of ulcers through diversion of the pancreatic juice from the duodenum even though such failure may be

lent increase in the bicarbonate concentration of the pancreatic juice as drainage progressed The increase in bicarbonate is not a constant finding since Heidenhain (40) obtained some specimens of juice from hypersecreting dogs which did not effervesce on addition of acid and some samples collected by Johnston and Ball had a pH as low as 7 16 The total base of the juice remains normal or nearly so (44) and because of this and the hypersecretion there is a substantial loss of base (Na, K, Ca) from the body which gives rise to changes in the blood that ultimately prove fatal

The most important blood changes are lowered plasma volume (dehydration) (43), decrease in chloride (44, 45, 46), decrease in bicarbonate (46, 47) and decrease in total base (45, 46) The pH of the blood may increase (43) or decrease (46) probably depending on the relative amounts of chloride and bicarbonate lost, excessive vomiting is common and this tends to increase the loss of chloride Hypersecretion of the stomach has been observed (47) in pancreatic fistula and this would add to the chloride loss when vomiting is present It may also account for the hypersecretion of the pancreas when HCl from the stomach enters the intestine In one group of animals studied by McCaughan (48) the blood chemistry remained approximately normal, the animals, nevertheless, died within 5 to 8 days (the usual survival time) apparently because of dehydration Changes in blood chemistry corresponding to those seen in experimental animals, but usually less severe, have been observed in human patients with pancreatic fistula (49)

Administration of sodium chloride or sodium bicarbonate intravenously or by mouth prolongs the life of experimental animals with pancreatic fistula but the ideal treatment is return of the secreted juice to the gastrointestinal tract For animals with an

POSSIBLE ROLE OF THE EXTERNAL SECRETION IN FAT METABOLISM

Pancreatectomy (36) or, in about half the experiments, ligation of the pancreatic ducts (15, 37) in dogs is followed by a disturbance in fat metabolism characterized by hypolipemia and deposition of excessive amounts of fat in the liver The question of whether these changes are the result of absence of pancreatic juice from the intestine or are due to lack of an internal secretion, the "lipocaic" of Dragstedt (38), has been the subject of an active controversy which this author will not attempt to resolve A critical review of the extensive literature on this subject has been prepared by McHenry and Patterson (39)

PANCREATIC FISTULA

The digestive disturbances that follow ligation of the ducts or removal of the pancreas are also observed when the pancreatic juice is diverted from the intestine through a complete fistula Such fistulas, however, give rise to other, more serious complications which make them unsuitable for the study of pancreatic function Within a few days after the fistula has been made the secretion becomes continuous and increases in amount and its composition changes The concentration of total solids falls to 1 or 2 per cent, chiefly owing to almost complete absence of protein (1, 40, 41, 42, 43) These changes are associated with changes in the microscopic appearance of the pancreas (40, 43), the acinous cells decrease in size and lose most of their granules The animal loses weight rapidly, refuses to eat and eventually dies

In animals with complete fistulas Johnston and Ball (44) found a decrease in chloride and an equiva-

lent increase in the bicarbonate concentration of the pancreatic juice as drainage progressed The increase in bicarbonate is not a constant finding since Heidenhain (40) obtained some specimens of juice from hypersecreting dogs which did not effervesce on addition of acid and some samples collected by Johnston and Ball had a pH as low as 7 16 The total base of the juice remains normal or nearly so (44) and because of this and the hypersecretion there is a substantial loss of base (Na, K, Ca) from the body which gives rise to changes in the blood that ultimately prove fatal

The most important blood changes are lowered plasma volume (dehydration) (43), decrease in chloride (44, 45, 46), decrease in bicarbonate (46, 47) and decrease in total base (45, 46) The pH of the blood may increase (43) or decrease (46) probably depending on the relative amounts of chloride and bicarbonate lost, excessive vomiting is common and this tends to increase the loss of chloride Hypersecretion of the stomach has been observed (47) in pancreatic fistula and this would add to the chloride loss when vomiting is present It may also account for the hypersecretion of the pancreas when HCl from the stomach enters the intestine In one group of animals studied by McCaughan (48) the blood chemistry remained approximately normal, the animals, nevertheless, died within 5 to 8 days (the usual survival time) apparently because of dehydration Changes in blood chemistry corresponding to those seen in experimental animals, but usually less severe, have been observed in human patients with pancreatic fistula (49)

Administration of sodium chloride or sodium bicarbonate intravenously or by mouth prolongs the life of experimental animals with pancreatic fistula but the ideal treatment is return of the secreted juice to the gastrointestinal tract For animals with an

incomplete fistula of the Pavlov type administration of sodium bicarbonate and a diet that does not stimulate excessive secretion such as bread and milk may be all that is required (42)

A summary of the results observed in experimental pancreatic insufficiency is presented in Tables II and III

TABLE II

Fun tional disturbances in pe im tal pancreatic i ufficien y

Proced re	Fun t onal chang s		
	Absorpt o deficit	D ode al lc r	F tty liv r
P creat ctomy	*100%* of animals (Sev rity v iable)	*R e*	92% (Drag tedt)
Ligatio of du ts	100% f a mals (Se rity v iabl)	33% (D ag t dt)	50% (D ag tedt)
T tal f t la	100% f animals (Sev rity v iabl)	(Limited by ea ly death) th rwi Pr bably 100% (Dr gstedt)	N e (50)
Mann William on Op ration	Som d ficit (No act data)	90 100%	(No data)

TABLE III

Blood chang s i p ime t l panc tic i s ffici cy

Proced r	Blood Changes			
	E zymes	S lts and W t	S gar	F t
Panc eatectomy	No change (possibl d cr as i blood amyla)	No hang	Hypergly mi	Hyp lipemia
Ligation of ducts	I ase i blood amyla e and lipas	N chang	N h g hyp gly emia a pos sible l t effe t	Hypolipemia in 50%
Tot l fistul	No hange	D re e in tot l ba e D eas in Cl *D c in HCO_3* Dehyd ation pH va iabl	N ch g	N change

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rats, guinea pigs and frogs (6) Baxter (5) found that secretion in the rabbit continues even after removal of the small intestine and after decapitation

The idea that the pancreas of the fasting dog does not secrete unless stimulated by means of food or other external agents has had to be modified in the light of subsequent work Boldyreff (7) observed secretion at intervals in the fasting animal associated with periods of secretory and motor activity in other organs of digestion which we now recognize as hunger periods This periodic secretion was studied thoroughly by Scott and his co-workers (8, 9, 10), they found that although periodic secretion did occur it was associated with hunger activity of the stomach only about half the time A peculiar result of their study was to show that the volume of the periodic secretion and frequency of secretory periods were modified by the type of fistula operation to which the experimental animal had been subjected The problem is to guess which type of fistula gave "normal" results The demonstrated fact that the presence of a pancreatic fistula may greatly change the behavior of the pancreas perhaps explains why Zucker, Newburger and Berg (11) found continuous secretion in their fistula dogs Babkin (25) has suggested that the so-called periodic secretion may not be true secretion but a pressing out of juice from the pancreas by periodic contraction of the ducts Such periodic contraction could readily be associated with periodic motor activity in the remainder of the gastrointestinal muscle

Continuous secretion is the rule in human patients with pancreatic fistula (12, 13, 14, 15, 16) and McClure (33) states that pancreatic enzymes are always found in the intestinal contents collected by intubation of normal subjects even when fasting

A more extended discussion of this subject is given by Babkin (2)

Chapter V

STIMULI FOR THE PANCREAS

THE early experiments of Heidenhain (1) indicated that the secretion of fluid and the secretion of enzymes by the pancreas were each controlled independently of the other The stimuli that evoke secretion of water and salts he called "secretory" stimuli and those that increase the output of enzymes he designated "trophic" stimuli Since each of these terms is commonly used in a different sense than proposed by Heidenhain, Babkin (2) has suggested substituting "hydrelatic" for "secretory" and "ecbolic" for "trophic" To avoid confusion Babkin's suggestion will be followed in this discussion All normal pancreatic juice contains enzymes but stimuli which increase the fluid output without increasing the output of enzymes are considered to be purely hydrelatic Purely ecbolic stimuli cause no visible secretion alone but they increase the enzyme content of the juice secreted in response to coincident hydrelatic stimuli

CONTINUOUS SECRETION

Claude Bernard (3) believed that the pancreas secretes only when stimulated as it is, for example, during digestion Heidenhain (4) noted, however, that the secretion was continuous in herbivorous animals but he agreed with Bernard that in the dog and cat and other carnivora there was no secretion without stimulation Proof has since been obtained that secretion is continuous in rabbits (5), white mice, white

because it complicates the study of other substances that must be used in watery solution The original studies are credited to Damaskin (Babkin, 19) who made his observations by introducing water into the stomach Crider and Thomas (23) compared the effect of distilled water introduced directly into the intestine with that of isotonic solutions of various substances such as sodium chloride and dextrose They found that whereas water alone was an effective stimulus, these isotonic solutions in water were not, at least their effect was insignificant compared to that of distilled water The juice secreted in response to water in the intestine has a relatively high enzyme content (51), water is, therefore, both an ecbolic and a hydrelatic stimulus

ACID

"Die Säure ist der starkste Erreger der Pankreassekretion"* (Babkin, 19) The original discovery of the effectiveness of acid in the intestine as a stimulus for the pancreas is credited to Becker (19, 24, 26) who showed that carbonated water is a more effective stimulus than pure water or solutions of neutral salts Subsequently Dolinsky (26) found that several common acids (hydrochloric, phosphoric, lactic, acetic) were effective stimuli As a matter of fact the effective stimulus is the hydrogen ion as Popielski (38) later demonstrated It was soon determined that the effect of acid was not abolished by section of the vagus nerves, removal of the spinal cord or removal of the celiac plexus or even by any combination of these operations (Popielski, 27) Furthermore the effect of acid was not diminished by the administration of atropine (28, 32) These

*Acid is the most powerful excitant of pancreatic secretion

CEPHALIC AND INTESTINAL PHASES OF PANCREATIC SECRETION

The pancreatic secretion that appears during digestion occurs in response to specific stimuli associated either with the act of eating or the presence of food in the gastrointestinal tract Proof that the act of eating serves as a stimulus to the pancreas was obtained in Pavlov's laboratory (17, 18) (see also Ivy, 22) by means of sham feeding, that is, feeding a dog provided with an esophagostomy so that the food never reached the stomach In these circumstances the pancreas begins to secrete within 1 to 1 1/2 minutes after feeding and continues for 12 to 20 minutes The volume secreted is small, only a few cc , but the concentration of enzymes is increased over that of the fasting secretion Tonkich (20) proved that the secretion is not due to gastric HCl entering the duodenum by observing it in dogs with the stomach separated from the duodenum, vagotomy or administration of atropine abolished the response to sham feeding the mere sight or smell of appetizing food may induce pancreatic secretion in human subjects (21)

Foods in the stomach do not, apparently, have any direct stimulating effect on the pancreas, that is to say there is no "gastric phase" of pancreatic secretion (22), but they become effective stimuli again when the gastric contents enter the intestine and initiate the "intestinal phase" (22) All the major constituents of the gastric chyme influence the secretory activity of the pancreas in one way or another after they enter the intestine, these include water, HCl, products of protein digestion, fats or fatty acids, soaps, and products of starch digestion

WATER AS A STIMULUS FOR THE PANCREAS

The action of water as a pancreatic excitant is important in spite of the fact that its action is slight,

because it complicates the study of other substances that must be used in watery solution The original studies are credited to Damaskin (Babkin, 19) who made his observations by introducing water into the stomach Crider and Thomas (23) compared the effect of distilled water introduced directly into the intestine with that of isotonic solutions of various substances such as sodium chloride and dextrose They found that whereas water alone was an effective stimulus, these isotonic solutions in water were not, at least their effect was insignificant compared to that of distilled water The juice secreted in response to water in the intestine has a relatively high enzyme content (51), water is, therefore, both an ecbolic and a hydrelatic stimulus

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*Acid is the most powerful excitant of pancreatic secretion

observations led to the discovery of secretin by Bayliss and Starling (29)

The experiments mentioned in the preceding paragraph were done on anesthetized animals In the absence of anesthesia acid causes a much more abundant secretion and the response is severely depressed (generally by more than 50 per cent) by either vagotomy (30) or atropinization (31, 45, 69) These facts may be explained in various ways but the simplest explanation is that acid, in addition to releasing secretin from the intestine, stimulates the pancreas through a nervous mechanism as suggested by Wertheimer (81) and by Fleig (55b)

The pancreatic juice secreted in response to acid in the intestine contains a relatively high concentration of bicarbonate (32), owing probably to the rapid rate of secretion, but only a small amount of protein, less in fact than that obtained with any other stimulus except pure secretin These facts have led some observers (33, 34) to the very reasonable conclusion, that the secretory response to acid is primarily concerned with the neutralizing function rather than the digestive function of pancreatic juice

The unquestioned fact that acid is the most potent stimulus for the pancreas along with the fact that HCl is usually present in the gastric juice has led logically to the conclusion that gastric HCl is the dominant factor in the regulation of pancreatic secretion in the course of normal digestion Attempts to verify this hypothesis by quantitative measurements have not been fully successful Popielski (38) has shown that the stimulating effect of acid on the pancreas is proportional to the hydrogen ion concentration of the solution as it enters the duodenum Thomas and Crider (36) studied the response of the pancreas to buffer mixtures of various acids and their sodium salts at different pH levels when introduced directly into the duodenum Most buffer

mixtures gave a good secretory response from the pancreas at pH 4 0 but a poor response at pH 4 5 Thomas (35) found that during digestion of meat the pH of the duodenal contents of dogs ranged from pH 3 0 to pH 4 8, the most frequent value being pH 4 0 Other foods cause a less acid duodenal content

Thomas concluded that during digestion of meat there is often sufficient acid in the intestine to be an effective stimulus for pancreatic secretion However, Pincus and Thomas (37) were unable to detect any significant positive correlation between the pH of the duodenal contents of dogs and the volume of pancreatic juice secreted during digestion of various types of food including meat Their observations support the contention of McClure (33) and Christiansen (34) that the importance of acid as a stimulus for the digestive secretion of the pancreas has been overestimated These authors studied pancreatic secretion in achlorhydric patients and found it to be essentially normal Considering all the evidence it appears doubtful that the conditions necessary for significant stimulation of the pancreas by means of acid in the intestine occur often enough during normal digestion to enable acid to play a dominant part in regulating the digestive secretion Furthermore the fact that acid is primarily a hydrelatic stimulus means that it can be of no great consequence in regulating the secretion of pancreatic enzymes under any circumstances

PRODUCTS OF PROTEIN DIGESTION

Recognition of the effectiveness of peptone in the intestine as a pancreatic stimulus is a comparatively recent development The earlier investigators (39, 40, 41) believed that it was ineffective alone and some even considered it an inhibitory agent (42) The important studies of McClure and his co-workers

(33) on the physiology of the human pancreas include what is probably the first demonstration of the effectiveness of protein digests as primary pancreatic stimuli Brjuchanow (40) had previously shown that peptone in the intestine increases the output of enzymes from the pancreas when the gland is stimulated by acid It was also known that peptones caused pancreatic secretion when they were placed in the stomach (31, 39, 43) but the secretory response was attributed either to water in the peptone solution or to HCl secreted by the stomach in response to peptone as a gastric stimulus In experiments on dogs

TABLE IV

Am nt and p ope ties of pancreatic sec etion produced by inj cting 20 cc of a s lution of variou protein dig sts into the int stin

Stimul s			Secretion					
	Total N	Per cent p oteo e	Num ber of mples	Vol per 10 min s mple	Sp g	Total N	Total N	Rati N secreted N injected
	mgm /cc			cc		mgm /cc	mgm / s mple	
C mmercial products								
Witte s peptone	5 95	(high)	13	2 93	1 0249	9 60	24 01	0 201
Bacto proton	7 92	81	31	3 06	1 0254	9 93	24 61	0 155
N o pepton	7 42	25	14	2 35	1 0209	8 20	17 71	0 133
Bacto pept e	8 26	6	42	1 83	1 0226	8 86	14 47	0 092
Amino acid powder (St arns)	6 46	0	11	1 08	1 0258	4 88	6 29	0 048
Labor tory p epa ti s								
Pep in digest of ca ein	4 0		21	2 27	1 0218	7 526	15 541	0 194
P p in trypsin digest of c sein	4 0		22	1 96	1 0244	8 71	14 30	0 176
Proteose fr m neo peptone	4 0	100	24	2 39	1 0205	6 48	13 88	0 175
P oteos from inte tinal cont ts	4 0	100	12	2 30	1 0202	6 33	13 27	0 164
Proteose from gastric conte ts	4 0	100	11	1 96	1 0199	7 00	12 55	0 156
†P T A d.ge t of ca ein	4 0	(tr)	10	1 99	1 0233	5 5	12 07	0 15

†P T A (P p in trypsin cid)
Average based o data f om 2 animal only

in which these factors were adequately controlled Thomas and Crider (44) found that various products of protein digestion including proteoses, peptones and some amino acids in the intestine has a moderate hydrelatic effect and a powerful ecbolic action on the pancreas Some of their data are given in Table IV Whether or not some native proteins are also effecttive has not been determined Egg albumen is known to be ineffective The ecbolic action of peptone as compared with some other stimuli is shown in Fig 14

Peptones cause no secretion in anesthetized animals nor in recently vagotomized animals but the response reappears within a few days after vagotomy, however, it remains permanently subnormal both as to volume and enzyme output (30) Atropine (0 2 to 0 3 mg /kg) increases the volume of secretion due to peptone and decreases the concentration of nitrogen but not necessarily the total nitrogen output (45) In normal dogs peptone causes depletion of the zymogen granules of the acinous cells as does stimulation of the vagus, this effect is not demonstrable in vagotomized dogs, at least during the first 24 hours after operation (46)

FATS, FATTY ACIDS AND SOAPS

For an adequate firsthand account of the early development of our knowledge regarding fat and fat products as stimuli for the pancreas the reader is referred to the several books and reviews by Babkin (2, 19, 47) He credits the first observations to Dolinsky (39) and Damaskin (48) in 1894 and 1895 respectively

The volume of secretion due to fat is less and the concentration of enzymes greater than in the juice secreted in response to HCl The effective stimulus may be the original neutral fat or it may be fatty

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TABLE IV

Amount and properties of pancreatic secretio produ ed by i fecting 20 cc of a soluti n of various prot in digests int the intestine

Stimulus			Secretion					
	Total N	Per cent prot ose	Num ber of samples	Vol per 10 min. sample	Sp g	Total N	Total N	Ratio N secreted N inj cted
	mgm /cc			cc		mgm./cc	mgm / sample	
Comm rcial p oducts								
Witte s peptone	5 95	(high)	13	2 93	1 0249	9 60	24 01	0 201
B cto proton	7 92	81	31	3 08	1 0 54	9 93	24 61	0 155
Neo pept ne	7 42	25	14	2 35	1 0209	8 20	17 71	0 133
Bacto pepton	8 26	6	42	1 83	1 0226	8 86	14 47	0 092
Amino acid powder (Stearns)	6 46	0	11	1 08	1 0258	4 88	6 29	0 048
Labo tory preparations								
Pepsin digest of cas in	4 0		21	2 27	1 0218	7 526	15 541	0 194
Pep in trypsin digest of casein	4 0		22	1 96	1 0244	8 71	14 30	0 178
Proteos from neo peptone	4 0	100	24	2 39	1 0205	6 48	13 88	0 173
Proteo e f om int stinal cont ts	4 0	100	12	2 30	1 0202	6 59	13 27	0 164
Proteo from gastric contents	4 0	100	11	1 96	1 0199	7 00	12 55	0 156
†P T A. digest of ca in	4 0	(trac)	10	1 99	1 0233	5 5	12 07	0 15

†P T A (Pepsin trypsin acid)
Average based on data fr m 2 animals only

acids or soaps produced by digestion Glycerol is not an effective stimulus (Babkin, 47) Tonkich (20) separated the stomach from the duodenum in pancreatic fistula dogs and found that neutral fat failed to stimulate the pancreas when introduced separately into either the stomach or duodenum She concluded that it was effective only when regurgitated from the duodenum into the stomach Her observations seemed to point to the rather improbable conclusion that the only site of effective stimulation is the mucosa of the pylorus itself In any case they indicate the neutral fat, when in the intestine only, is not an effective stimulus for the pancreas

There is no doubt about the effectiveness of fatty acids (31, 49) or soaps (50) These agents were effective in the experiments just described (20) when placed in the separated duodenum Soap introduced directly into the intestine causes a free flow of pancreatic juice which may be as abundant as that caused by HCl The specific gravity and nitrogen content are, however, always greater than in secretion due to acid When the soap is mixed with an equivalent amount of HCl to release the fatty acids the result is not changed with respect to either volume or concentration (51)

The question of the mechanism through which fatty acids and soaps stimulate the pancreas is one of the most puzzling in all pancreatic physiology Their effect is in no wise modified by section of all the extrinsic nerves (30, 52), it is, on the other hand, greatly reduced or abolished by atropinization (31, 45, 53) When soap is introduced into an intestinal loop and later withdrawn it contains a pancreatic secretagogue, effective on intravenous injection, which causes secretion of pancreatic juice similar in its properties to that caused by soap in the intestine The effect of this secretagogue is greatly reduced by giving atropine to the test animal (54), it is

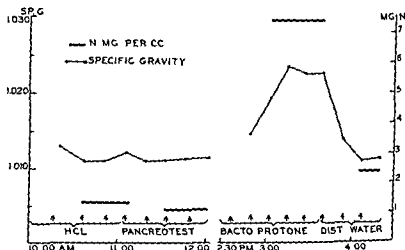


Figure 14

Graphic protocol of a typical experiment showing specific gravity (round dots) and total nitrogen in mg /cc (heavy horizontal bars) of pancreatic juice secreted by a chronic fistula animal in response to various stimuli including a commercial peptone (bacto-protone) The small arrows mark the times at which the various stimulating substances were given Pancreotest (commercial secretin) was given by vein the others were placed in the duodenum (From Thomas and Crider (44) Am J Physiol)

evidence in support of the above supposition, but undoubtedly during the absorption of fat in the intestine some substances are formed in or released from the mucous membrane, which when carried by the blood to the pancreas, stimulate its secretory nerves "

A summary of some of the properties of pancreatic juice obtained with different stimuli is given in Table V, prepared by Dr Joseph Conly from an analysis of our laboratory records

CARBOHYDRATES

The carbohydrates have generally not been regarded as effective stimuli for the pancreas except indirectly through changes in blood sugar concentration Hebb (57), working in Babkin's laboratory found that the enzyme concentration in the continuously secreted pancreatic juice of rabbits varied directly with the concentration of sugar in the blood Other studies of the relation of blood sugar to pancreatic secretion have given conflicting results, some supporting Hebb s conclusions (58, 59, 60), others indicating a contrary relationship (61, 62, 63) Ferrari (64) found that both hypoglycemia, induced by insulin, and hyperglycemia increased the output of enzymes from the pancreas of chronic fistula dogs (For additional references see Babkin, 2) Recent experiments by Thomas and Crider (65) (Fig 15) and by Friedman and Snape (82) performed in our laboratory support the view that hypoglycemia tends to increase rather than decrease the enzyme output from the secreting pancreas The situation is complicated by differences in experimental conditions and possible species variations but in general it appears that a high blood sugar concentration favors secretion of enzymes through a local action on the gland or on the nerve endings within the gland while a low blood sugar has the same effect on the enzyme

therefore not ordinary secretin These observations recall the experiments of Fleig (55a) with extracts of intestinal mucosa made with solutions of soap He obtained a secretagogue which he called "sapocrinin" that differed in many respects from secretin One *difference was that its effect* was diminished by atropine

In his comment on Fleig's observations Pavlov (56) remarked that, "The immediate excitant of the pancreas is a substance resembling, but not identical with, acid 'secretin'" Babkin (2) has carried this idea farther in the following words "Thus other substances that might be formed from the intestinal mucosa are unable to stimulate the acinous cells of the gland if the secretory nerves through which they normally act are paralyzed by atropine One of these substances might be choline, for fat may be absorbed in the form of lecithin, Best and his co-workers emphasized the important part played by choline in the metabolism of fat There is no experimental

TABLE V

Aver ge specific gravity total nitrogen and p otein content of dog's pancr atic juice secreted in response to different stimuli

Stimulus	Number of Experiments	Jumber of dogs	Average Sp Gr	Total Nitrogen (calculated)	Total Protein (calculated)
				mg /cc	per cent
Hydrochl ri Acid	44	15	1 0105	1 75	1 1
Peptones	79	19	1 0200	7 40	4 6
Soap	16	6	1 0141	3 90	2 4
Pancreotest (comm rcial ecretin)	12	7	1 0110†	2 05	1 3
Gl tamic Acid	4	2	1 0098‡	1 22	0 76
Secretin (Friedman)	26	6	1 0103	1 64	1 0

Each experiment rep ese t from 2 t 7 sampl
†Difference fr m Secretin (Friedman) and HCl not significant. Includ s several experi m nts on one dog which gave excessively high values with ll stimuli
‡Diffe ence from HCl probably not significant

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A summary of some of the properties of pancreatic juice obtained with different stimuli is given in Table V, prepared by Dr Joseph Conly from an analysis of our laboratory records

CARBOHYDRATES

The carbohydrates have generally not been regarded as effective stimuli for the pancreas except indirectly through changes in blood sugar concentration Hebb (57), working in Babkin's laboratory found that the enzyme concentration in the continuously secreted pancreatic juice of rabbits varied directly with the concentration of sugar in the blood Other studies of the relation of blood sugar to pancreatic secretion have given conflicting results, some supporting Hebb s conclusions (58, 59, 60), others indicating a contrary relationship (61, 62, 63) Ferrari (64) found that both hypoglycemia, induced by insulin, and hyperglycemia increased the output of enzymes from the pancreas of chronic fistula dogs (For additional references see Babkin, 2) Recent experiments by Thomas and Crider (65) (Fig 15) and by Friedman and Snape (82) performed in our laboratory support the view that hypoglycemia tends to increase rather than decrease the enzyme output from the secreting pancreas The situation is complicated by differences in experimental conditions and possible species variations but in general it appears that a high blood sugar concentration favors secretion of enzymes through a local action on the gland or on the nerve endings within the gland while a low blood sugar has the same effect on the enzyme

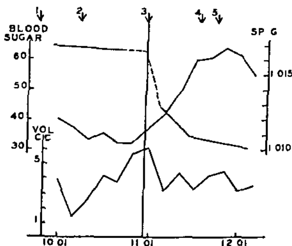


Figure 15

Curves showing the effect of insulin-induced hypoglycemia on the protein (enzyme) content of pancreatic juice in terms of specific gravity, secreted in response to continuous secretin Upper curve, blood sugar in mg /100 ml , middle curve, specific gravity of the pancreatic juice, lower curve, volume of juice secreted in 10 minutes Experiment on a chronic fistula dog Insulin (15 units intravenously) given at 11 01 (From Thomas and Crider (65) Proc Soc Exper Biol and Med)

output through stimulation of medullary centers provided the experimental subject is not anesthetized In the anesthetized animal either the central effect is reversed or the local effect, in this case a decrease in enzyme output, predominates

There is nevertheless a possibility that some carbohydrates act as specific stimuli for the pancreas when present in the intestine, in a manner comparable to the effect of the other foodstuffs Babkin and Savich in 1921 (quoted by Babkin, 2) found that adding cane sugar to an acid solution used as a

stimulus for the pancreas greatly increased the tryptic power of the secreted juice Similar results with starch were reported by Harper and Vass (66) Recently Thomas and Crider (67) have investigated the effect of placing various carbohydrates in the intestine of chronic fistula dogs which were being given secretin by continuous intravenous injection Under these conditions several carbohydrates (partly digested starch, maltose, lactose) regularly caused an increase in nitrogen output from the gland either by increasing the rate of secretion or the concentration in terms of total nitrogen or both These effects were completely abolished by atropine In the experiments by Miss Hebb, referred to previously, the ecbolic effect of an increase in blood sugar concentration was likewise abolished by atropine In our experiments some carbohydrates had no effect or the results were doubtful, these included undigested starch and some commercial dextrins All the effective carbohydrates were to some extend absorbable, consequently there was a measurable though slight increase in blood sugar in every instance in which there was an increase in nitrogen output from the pancreas Before it can finally be concluded that these agents acted as pancreatic stimuli in the intestine it will be necessary to measure the effect of such increases in blood sugar concentration as occurred to determine what part, if any, they played in bringing about the observed results This has not yet been done Whether they act locally or from the intestine it is evident that the carbohydrates are mainly ecbolic rather than hydrelatic stimuli They are not, of themselves, able to cause secretion in a resting gland

BILE AS A PANCREATIC STIMULUS

Mellanby (68), in 1926, reported some experiments which seemed to indicate that bile in the

intestine acts as an effective stimulus for pancreatic secretion by increasing the absorption of secretin from the intestinal mucosa His experiments were performed on anesthetized cats which had been under the influence of urethane for several hours Subsequently Lueth and Ivy (69) and still later Dragstedt and Woodbury (70) compared the pancreatic secretion in chronic fistula dogs in which bile was excluded from the intestine with the secretion in a control group in which bile was present, neither found any significant difference On the contrary, Lueth and Ivy noted that bile in the intestine sometimes depressed pancreatic

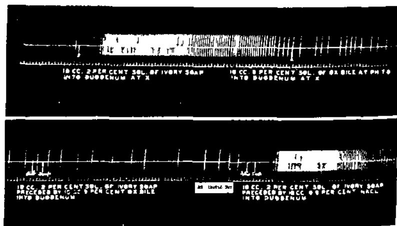


Figure 16

Inhibitory effect of bile on pancreatic secretion Kymographic record showing drops of pancreatic juice (as vertical lines) secreted in response to placing in the intestine soap alone bile alone bile and soap and finally soap alone after a recovery period of 25 minutes There was little or no extra secretion with bile alone or with soap when bile was also present Chronic fistula dog (From Thomas and Crider (71) Am J Physiol)

secretion Thomas and Crider (44) were unable to detect any stimulating effect on the pancreas when bile was introduced into the intestine in unanesthetized dogs and they subsequently (71) found that bile profoundly inhibits the secretory response to peptone and soap in the intestine (Fig 16) and to some extent also the response to HCl

In a series of experiments on anesthetized animals we (51) attempted to repeat Mellanby s observation, but were unable to duplicate his results except under special conditions When the animals were in a state of "shock" following prolonged anesthesia or when the anesthesia was pushed to the point of causing a profound fall in the blood pressure or if the blood pressure was lowered by giving nitrites in large doses, bile in the intestine caused the pancreas to secrete, sometimes at a very rapid rate otherwise the results were negative Results were the same in dogs as in cats, hence the conflict between Mellanby's results and those of subsequent investigators can not be explained on the basis of species differences This conclusion is further supported by some experiments of Florey and Harding (72) in unanesthetized cats They found that a portion of the pancreas transplanted by the method of Farrell and Ivy (73) secreted more freely after ligation of the bile ducts than before

The experimental evidence indicates that although bile in the intestine may promote absorption of secretin and thus stimulate the pancreas under certain experimental conditions it does not do so normally, at least not in sufficient amounts to offset the more characteristic inhibitory effect Nevertheless it may be unwise to conclude that bile in the intestine is without effect on the pancreas The consistent "inhibitory" action and the occasional secretory effect are probably not what they seem they may be manifestations of an action on the secretory mechanism

which would appear useful and consistent if properly understood

OTHER PANCREATIC STIMULI EFFECTIVE IN THE INTESTINE

Of the numerous foreign substances which are effective as pancreatic stimuli in the intestine such as alcohol, ether, chloral hydrate, mustard oil, croton oil, pepper extract, formaldehyde, magnesium sulfate, etc (for literature see Babkin, 19), only ether and magnesium sulfate have been put to practical use

The effect of ether was described by Claude Bernard and it has since been used occasionally as a test for pancreatic function in human subjects Magnesium sulfate has been used in a similar manner (33) to obtain bile and pancreatic juice for clinical analysis

DRUGS AND OTHER STIMULI EFFECTIVE IN THE BLOOD STREAM

Most substances that lower blood pressure either initiate pancreatic secretion or enhance the response to other stimuli such as secretin Agents effective in this way are certain commercial peptones, e g Witte's peptone, histamine and the nitrites (74, 77) In the case of the nitrites at least, the secretory effect is almost entirely due to lowering the threshold of the pancreas to secretin stimulation It is practically ineffective in animals from which the small intestine has been removed (51)

Methyl and dimethyl guanadine, which raise the blood pressure, also stimulate the pancreas (75) Substances of this nature and histamine or histamine-like substances are probably responsible for the secretory effect of non-specific tissue extracts

Acetylcholine, acetylbetamethylcholine chloride

(Mecholyl, Merck), cholıne, physostigmıne, neostygmıne, and pilocarpıne act as ecbolic stımuli for the pancreas in appropriate doses Since their hydrelatıc effect ıs small they are somewhat unreliable when used alone, causıng at most only a small volume *of hıghly concentrated secretıon* They work best ın combinatıon wıth hydrelatıc stımuli, for example, secretın, and in these cırcumstances they increase the concentration of enzymes ın the secretıon The use of Mecholyl ın a dose of 15 mgm given subcutaneously has been proposed as a supplement to the secretın test for pancreatıc function (76)

Atropıne, in additıon to the actıons mentıoned ın the precedıng sections, paralyzes the secretory endıngs of the vagus ın doses of 0 2 to 0 3 mg /kg in dogs Hyoscyamine has an identıcal actıon ın somewhat smaller doses (0 15 to 0 2 mg /kg) (45) In very large doses atropine may actually ıncrease the volume of pancreatıc secretıon and moderate doses may have a sımilar actıon ın the presence of certaın stımulı, for example, peptone (45) or mılk and cream (20) The characteristıc effects of faırly large doses of atropıne as observed in experimental anımals, namely, decrease ın volume, and ın the concentration of *enzymes ın the pancreatic juıce are not obtaıned* with therapeutıc doses ın human subjects (78)

Epınephrıne (79) and other sympathomimetıc drugs generally ınhıbıt pancreatıc secretıon They owe at least a part of theır effect to the fact that they decrease blood flow through the gland Ephedrıne (80) seems to be particularly effectıve and has been recommended for decreasıng the volume of secretıon ın pancreatıc fistula

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Chapter VI

SECRETIN AND PANCREOZYMIN

THE events that led to the discovery of secretin have been mentioned in the preceding chapter Bayliss and Starling (1a) conceived the idea that acid in the intestine liberates a hormone from the mucous membrane that is carried by the blood to the pancreas where it acts as a secretory stimulus This hormone they called secretin Their announcement, in 1902, was followed by a long and sometimes bitter controversy but the accumulated evidence has supported their hypothesis in all its major aspects The literature dealing with this phase of the subject has been reviewed by Still (2) in his valuable paper on "Secretin" and even more thoroughly by Babkin (3)

PROOF OF A HUMORAL MECHANISM FOR PANCREATIC SECRETION

Very soon after the discovery of secretin the possibility of hormonal stimulation was put to a crucial test in several laboratories *Within the year Wertheimer* (4) had " succeeded in demonstrating the presence of secretin in the blood flowing from a loop of intestine into which acid had been introduced " By means of cross-circulation experiments, in which the blood of one animal was made to circulate in the body of another, Enriquez and Hallion (5) and Fleig (6) and later Matsuo (7) proved that when HCl was placed in the duodenum of one animal, the donor, the pancreas of the second animal, the recipient, also secreted In 1926 Farrel and Ivy (8) succeeded in transplanting the

tail of the pancreas of a lactating bitch into the tissue of a mammary gland where it received an entirely new blood supply and was, of course, completely separated from its original nerve supply The continuous secretion of the transplant was augmented when the animal was given food, water or acid solution, proving that some stimulating agent was carried to the gland by the blood

The following year Houssay and Molinelli (9) transplanted the pancreas together with the duodenum of one dog into the neck of a second animal by anastomosing the blood vessels Both animals were anesthetized In this situation the pancreas secreted when acid was put into the duodenum of the second dog A little later Necheles and Lim (10) separated gastric and pancreatic secretagogues from the blood of dogs by circulating the blood of the living animal through a dialyzing apparatus The pancreatic secretagogue proved to be present even in fasting animals but was increased in amount after feeding

DISTRIBUTION OF SECRETIN

As originally prepared by Bayliss and Starling (1a) secretin differed only quantitatively from other tissue extracts, many of which contain pancreatic secretagogues As methods of extraction improved it became possible to prepare extracts of the intestinal mucosa by procedures which did not yield pancreatic secretagogues when applied to other tissues and organs (7, 11, 12, 13, 14) It is now generally understood that secretin is present only in the mucous membrane of the small intestine Several investigators (11, 12, 13, 14) have shown that the amount of secretin that can be extracted from the intestine is greatest in the duodenum and becomes progressively less the greater the distance from the pylorus

There are, apparently, some differences in the distribution of secretin within the gut in different species since Mellanby (11, 12) found that secretin was practically limited to the duodenum in the cat but could be obtained from the upper ten feet of jejunum in the pig, in the goat he found it even in the ileum Friedman and King (15) studied the distribution of secretin in the dog and concluded that it was present to some extent throughout the length of the small intestine in this animal They estimated the secretin content of the terminal 10 cm of ileum to be about 2 per cent of the amount in the first 10 cm of duodenum Results of their study of the upper 60 cm of intestine are shown in Table VI An interesting fact brought out by these

TABLE VI

Average secretin content of the upper small intestine in 14 dogs

Distance from pylorus	Weight of intestine	Weight of extract	Secretin Content		
cm	gm /cm intestine	mg /gm intestine	units/mg extract	units/gm intestine	units/cm. intestine
0 10	1 7	2 07	0 45	0 93	1 61
15 25	1 5	2 53	0 35	0 88	1 47
50 60	1 3	2 90	0 15	0 43	0 57

To avoid loss in purification the first acetone precipitate was used (see under Preparation of Secretin below) It was relatively free from vasodilator substances

data is that the differences in secretin content per gm of tissue at various levels of the intestine are considerably less than the differences per cm of length The latter comparison is the one commonly made but it exaggerates the differences because of the lesser weight of the intestine per cm at lower levels

PREPARATION OF SECRETIN

The numerous methods that have been used for extracting and purifying secretin have been reviewed by Still (2) and by Greengard and Ivy (16) In most procedures the initial process is extraction of the mucosa of the small intestine with acid, usually HCl Originally (1a) this was done with the aid of heat after grinding the mucosa with sand but after Matsuo (7) showed that secretin could be extracted from the living intestine less drastic procedures were adopted Luckhardt with Barlow, Weaver and Koch (17, 18) found that when the lumen of the freshly excised intestine was filled with 0 2 to 0 4 per cent HCl, after 20 to 30 minutes the acid contained non-toxic secretin They greatly simplified the further purification by showing that secretin could be precipitated by saturating the acid extract with NaCl

This basic procedure for obtaining a non-toxic, crude secretin has been modified only in technical detail by most subsequent workers The freshly excised intestine is generally turned inside out (16) and immersed in 0 4 per cent HCl for 30 minutes after which the acid extract is saturated with NaCl The resulting precipitate, designated "salt cake," is separated by suction filtration The precipitate obtained by this or similar procedures has provided the starting point for further purification of secretin by several laboratories (15, 16 19)

The Hammarstens with Jorpes, Agren and Wilander (20, 21) obtained a crystalline preparation of secretin by methods somewhat different from those just described They extracted the fresh hog intestine with N/20 sulfuric acid and precipitated the active material from the extract with sulfate of mercury The mercury was later removed by means of hydrogen sulfide After further purification the active material was converted into a picrolonate and crystallized from

solution in anhydrous pyridine Their product had a high potency (threshold dose for the cat--0 0042 mg) and was believed to represent the picrolonic acid salt of the pure hormone *

Greengard and Ivy (16) also prepared a secretin picrolonate that could be crystallized from solution in pyridine Their crystalline product had about the same potency as that obtained by the Hammarsten group but differed from it in certain important respects, for example, it contained much more picrolonic acid Subsequent work (Greengard et al , 22) has shown that this product was a complex of secretin-pyridine picrolonate and could not be distinguished by crystallographic methods from pyridine picrolonate The significance of these findings and their bearing on the previous work of the Hammarsten group is not clear, the latter also used pyridine in their final crystallization Until the same tests are applied to their product as were applied to that of Greengard and Ivy it will not be known whether or not secretin has been obtained as a pure crystalline salt

At the outbreak of World War II when commercial secretin (see footnote, this page) became unavailable an investigation was undertaken in the physiological laboratory, under the direction of Dr M H F Friedman, of various methods of preparing secretin in the hope of developing a simple, reliable procedure that would yield a product sufficiently pure for clinical use and for most experimental purposes We were interested primarily in supplying our own laboratory but we also had in mind the need for a domestic source of secretin for use as a test of pancreatic function in

*These investigators also developed a less pure amorphous preparation of secr tin for clinical use which was made commercially in Sweden and sold in this country under the trade name "Pancreotest "

patients The method that was finally adopted consistently yields a potent preparation which is not toxic and apparently not antigenic It is free from other known intestinal hormones such as cholecystokinin and enterocrinin and has proved to be entirely suitable for the purposes for which it was developed The procedure as described by Dr Friedman (23) is given in the next paragraph

"The salt cake obtained by saturating the HCl extract of intestinal mucosa with NaCl is dried by suction filtration until it contains less than 40 per cent moisture A weighed amount of the dried salt cake is suspended in five parts (by weight) of absolute methanol After standing 24 hours in the cold the material is separated by filtration and the residue reextracted with 2 5 parts of methanol Both filtrates are combined and exactly three volumes of absolute acetone is added The acetone precipitate of the methanol filtrate is collected by suction filtration and dried with acetone and ethyl ether The precipitate is then suspended in 100 parts of distilled water acidified by the addition of two parts glacial acetic acid Insoluble material is removed by filtration in the cold To the filtrate is added enough of a 100 per cent solution of cold trichloracetic acid reagent to make a final concentration of 10 per cent The resulting fine precipitate is collected by centrifugation at low speed for 20 to 30 minutes After the precipitate is washed with absolute acetone and ethyl ether it is suspended in 50 parts of normal butyl alcohol The insoluble material remaining after agitation for three hours is recovered by centrifugation and washed with absolute acetone and ether The dried precipitate is then dissolved in 5 to 10 parts of distilled water made slightly acid with HCl and material remaining undissolved is discarded The liquid is then poured into lyophilization flasks of suitable size, frozen, and lyophilized in the usual manner Instead of lyophilization the active secretin fraction

may be recovered by the addition of absolute acetone to make a concentration of 85 per cent However the lyophilized product is to be preferred because it is much more readily soluble Both the lyophilized and the acetone-precipitated preparations have been found to yield similar results in human and animal experiments "

ASSAY OF SECRETIN PREPARATIONS

The standardization of secretin preparations should include the following three steps (1) definition of a secretin unit, (2) assay, in terms of the unit adopted, of a stable preparation for use as a standard, (3) comparison of the unknowns with the standard Ivy and co-workers (24) have defined a unit, now commonly known as the Ivy dog unit, as "The amount of dried material in solution which when injected intravenously" into an anesthetized dog "will cause a 10 drop increase in rate of flow of pancreatic juice within a ten minute period following the time of injection as compared with the preceding ten minute period " Between 0 075 and 0 08 mg of the picrolonate as prepared by Greengard and Ivy (16) or 0 5 mg of Pancreotest (25) equals one Ivy dog unit Wilander and Ågren (26) have defined a different unit using cats as test animals The Agren cat unit is equal to 0 004 mg of the crystalline secretin picrolonate as prepared by the Hammarsten group (20) or to 0 031 mg of Pancreotest Later Agren and Lagerlof (27a) recommended the use of 16 cat units per kilo body weight as a desirable dosage for measurement of pancreatic function in human subjects and this amount (16 cat units) was adopted by the manufacturers of Pancreotest as a clinical unit Greengard and Ivy (16) originally estimated the Ågren cat unit to be 1/20 the Ivy dog unit but this estimate was later corrected to 1/16 (25) As a consequence it happens that the Ivy unit and the Swedish

clinical unit are identical, a fortuitious but fortunate circumstance which seems to warrant adhering to this unit, whatever it may be called, as a universal standard *

The method of assay described by Ivy and his co-workers (24) is not suitable for routine use because of the large number of animals required for each determination In the work of Friedman in our laboratory (15, 23), referred to above, this method was used to determine the potency of a stock preparation to serve as a primary standard which was then used for the assay of secondary standards In order to conserve the primary standard most routine assays are made with secondary standards To compare either standard with a preparation to be tested, a dog weighing between 9 and 15 kilos is anesthetized with sodium pentobarbital and the major pancreatic duct cannulated after the accessory duct has been ligated The animal is given a "priming" dose of some potent secretin preparation to clear the ducts of accumulated secretion and insure patency of the cannula, etc After the response to this dose has subsided and the secretion has stopped or become constant, alternate varying doses of the standard and unknown are given at 10-minute intervals and the resulting secretion recorded with a drop recorder The dosage of the unknown is adjusted after the first trial so as to cause rates of secretion

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*Since this was written Burn and Holton (J Physiol 107 449 1948) have again emphasized the need for an international secretin unit and an international standard preparation to be used in the assay of new products For the latter purpose they suggest using the whole mucosa of the upper five feet of dogs small intestine in the form of an acetone-dried powder They describe bioassays of both secretin and pancreozymin preparations using the powdered mucosa as a standard

approximating those obtained with the standard Curves are plotted from the results obtained with the standard and the unknown showing the number of drops of secretion per 10 minutes as abscissas and the dosage of the secretin preparations are ordinates Doses of the standard are plotted in units and the unknown in milligrams Typical curves are reproduced in Fig 17

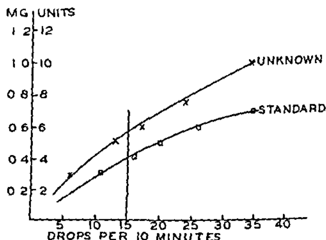


Figure 17

Curves obtained in the assay of a secretin preparation The *ordinate corresponding to* 15 drops *intersects the curve of the unknown* at 0 55 mg and the curve of the standard at 4 0 units

To calculate the potency of the unknown from these data, doses of the standard and unknown which yield the same rate of secretion, as indicated by the curves, are compared For example, in the assay *illustrated* in Fig 17, 0 55 mg of the unknown caused the same rate of secretion (15 drops in 10 min) as 4 0 units of the standard The unknown therefore contained $\frac{4\ 0}{0\ 55}$

or 7 2 units per mg From four to six such assays are made, each on a different animal and the results averaged as shown in Table VII

TABLE VII

Assay of secretin preparation WI12

Dog No	Dose required to produce 15 drops of pancreatic juice		Units per mg of Unknown
	Standard	Unknown (WI12)	
	units	mg	
46 12	6 5	0 95	6 8
46 13	2 0	0 25	8 0
46 25	5 7	0 75	7 6
46 26	4 5	0 70	6 4
46 27	4 0	0 55	7 2
		Average	7 2

CHEMICAL NATURE OF SECRETIN

Most observers agree that secretin is destroyed by proteolytic enzymes (28 29, 30, 31, 32, 33) and Greengard, Stein and Ivy (34) have shown that it is destroyed by an enzyme (secretinase) found in blood and urine Ågren (28) and Ågren and Hammarsten (29) find their cyrstalline material to be a polypeptid with a molecular weight of 5000, its amino acid constitution has been in part determined (28, 35) It is digested by aminopeptidase but not by carboxypeptidase As much as 40 per cent of the amino acids constituting the molecule may split off by aminopeptidase without altering the activity

Carlson (32), Still (2) and Ivy (16) and their coworkers doubt that the secretin in their preparations is a polypeptid Their purified material has a low nitrogen content (around 7 per cent) and combines with too much acid to be a substance of high molecular

approximating those obtained with the standard Curves are plotted from the results obtained with the standard and the unknown showing the number of drops of secretion per 10 minutes as abscissas and the dosage of the secretin preparations are ordinates Doses of the standard are plotted in units and the unknown in milligrams Typical curves are reproduced in Fig 17

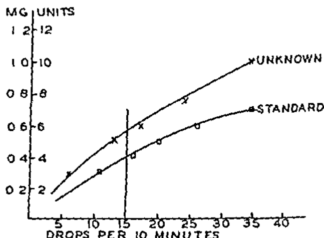


Figure 17

Curves obtained in the assay of a secretin preparation The ordinate corresponding to 15 drops intersects the curve of the unknown at 0 55 mg and the curve of the standard at 4 0 units

To calculate the potency of the unknown from these data, doses of the standard and unknown which yield the same rate of secretion, as indicated by the curves, are compared For example, in the assay illustrated in Fig 17, 0 55 mg of the unknown caused the same rate of secretion (15 drops in 10 min) as 4 0 units of the standard The unknown therefore contained $\frac{4\ 0}{0\ 55}$

blood pressure, are now known to be due to nonspecific tissue extractives that are present in crude secretin Some have been shown to indicate the presence of other specific hormones, the better known of these are cholecystokinin (52) which contracts the gall bladder and enterocrinin (53) which stimulates the secretion of succus entericus Many of the effects of crude secretin have never been explained and provide a fruitful field for further study, especially in the search for new duodenal hormones

Purified secretin, so far as we know at present, acts only on the pancreas and liver and, possibly, the intestinal glands On the pancreas it acts as a hydrelatic stimulus It has been suggested that secretin exhibits some ecbolic action as well but the evidence is at best inconclusive Barrington found that if the pancreas of a cat is made to secrete continuously by repeated doses of secretin, the concentration of enzymes falls to a constant low level after a time but may be increased momentarily by increasing the dose of secretin He attributed the increase to an ecbolic action of secretin In a large series of similar experiments on dogs Wang, Grossman and Ivy (51) saw an increase in enzyme output only once on increasing the dosage of secretin They explained that " this rather insignificant increase might be attributed to a 'washing out' from the gland of the enzyme which was presumably accumulated in the glandular passages of acini which were inactive before the increase of secretin stimulation took effect, the process therefore being a passive one "* Whichever interpretation ultimately proves to be correct it is certain that secretin possesses less ecbolic action than any other known stimulus except HCl to which it is almost exactly equal in this respect

*In 1942 Thomas (Federation Proc 1 265) proposed a similar explanation for Barrington s results

weight (16) The crystalline material of Greengard and Ivy gives a positive Biuret test but is negative to the Millon, ninhydrin, and Hopkins-Cole reactions Hammarsten confirms the absence of the ninhydrin reaction in active secretin but finds it present in preparations that have been inactivated (36) It seems clear that chemically different products with similar or identical physiological properties have been isolated from the intestine and called "secretin" Greengard and Ivy suggest that the secretin of the Hammersten group may have the same relation to their (Greengard and *Ivy's) product that thyroglobulin has to thyroxin*

All observers agree that secretin is a basic substance that readily forms salts with free acids According to Ågren (37) its isoelectric point and point of minimum solubility is at pH 7 8 It is relatively stable in acid solution and absolutely stable in the dry state when pure It is not destroyed by boiling or autoclaving at 100°C for 30 minutes but is quickly destroyed by N/5 acid or alkali at boiling temperature (32)

PHYSIOLOGIC ACTIONS OF SECRETIN

The original crude secretin of Bayliss and Starling, besides increasing the flow of pancreatic juice, increased the volume (38) and pepsin content (39, 40) of gastric juice and stimulated secretion of succus entericus (41), Brunner's gland secretion (42, 43), bile (1) and urine (44), it has also been reported to increase the flow of saliva, lachrymal fluid and lymph (2) It caused contraction of the gall bladder (45), duodenum (46) and spleen (47) Other secretin preparations have been said to lower blood sugar (48, 49) and to increase the red-cell (50) and hemoglobin content of the blood All the early secretin preparations caused a marked fall in blood pressure

Many *of these actions, particularly the fall in*

The purest secretin available increases the secretion of bile by the liver although it does not cause contraction of the gall bladder (55) Only Takacs (56) claims to have prepared secretin which did not stimulate bile formation and his method of observing bile flow (chronic biliary fistula) may not have been adequate Secretin causes only a moderate increase in bile flow (57, 33) and none at all if the liver is already secreting vigorously (Fig 18) The maximal rate of bile flow that can be induced by secretin is only about one-tenth the rate that can be attained by administering bile acids and it cannot be increased by increasing the dose of secretin (33) The increase in the amount of bile formed is usually less than the increase in the amount of pancreatic juice secreted in response to a given dose of secretin (57, 33) and the latent period of the liver is longer than that of the pancreas (58) Most of these facts are illustrated in Fig 18 which was kindly prepared for use in this discussion by Dr Friedman from some of his studies *

Friedman and Snape (59) found that different "secretins" (intestinal extracts) differed in the ratio of their choleretic effects to their secretory effects on the pancreas All potent secretin preparations were effective in stimulating the liver but not all extracts

←

the common bile duct time in 20 seconds S — point of administration of secretin The figures indicate the number of drops of pancreatic juice and of bile secreted during a 10 minute period immediately preceding and following the injection of secretin In the experiment shown on the bottom record the dog was given 1 cc of 20% decholin intravenously 2 hours 40 minutes before the secretin injection Records from an acute experiment with Nembutal anesthesia cholecystectomy cystic duct and accessory pancreatic duct ligated

*With I J Pincus W J Snape and Elizabeth King

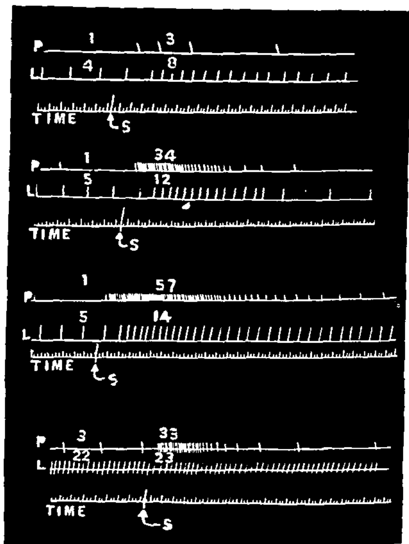


Figure 18

Copy of several kymographic records showing the effect of various doses of a secretin preparation on secretion from the pancreas and liver P — drops of pancreatic juice from the main pancreatic duct L — drops of bile from
⟶

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Figure 18

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venously 16 cat units (1 clinical unit) per kilo body weight of the secretin preparation and continued to collect the duodenal and gastric contents over a period of two hours Volume, bicarbonate, amylase and trypsin and, in later studies (55, 64, 66), bilirubin were determined on the material collected from the duodenum Characteristic deviations from normal values were found in acute and chronic pancreatitis and in cancer of the pancreas The most significant changes were in the absolute and relative amounts of the two enzymes, volume and bicarbonate were frequently within the normal range even in severe pancreatic disease (see Chapter VIII) This test has since been used by numerous observers abroad and in this country especially by Diamond and his co-workers (67)

Because secretin may not specifically stimulate enzyme output it has been suggested that an ecbolic stimulus should be added to the test for pancreatic function Comfort and Osterberg (68) have proposed using secretin and Mecholyl and have shown that the latter increases the enzyme output Since Frisk and Welin (69) and Lagerlöf and Welin (70) have shown that insulin increases the enzyme output during secretin stimulation, Friedman and Snape (71) have explored the possibility of using insulin as an accessory stimulus for enzyme production They found that in all cases in which the enzyme output was below normal this condition was evident from the secretin test alone and it did not appear that the use of insulin yielded any additional information of diagnostic value The same will probably prove to be true for Mecholyl, pancreozymin and other agents proposed to increase enzyme output

The secretin test has been found also to yield useful information regarding the functional state of the gall bladder (27b, 55, 67, 72) It will be recalled that although secretin increases the output of bile from the liver it does not cause the gall bladder to empty,

that were effective on the liver were effective on the pancreas They suggest that there may be a specific liver hormone (hepatocrinin) in the intestine

The effect, if any, of pure secretin on the intestinal glands remains to be determined The Hammarsten group thought their crystalline secretin increased the output of succus entericus but Nasset (60) states that they now agree with him that their method of study was not adequate to prove a secretagogue action A sample of secretin prepared for clinical use by Friedman's method " failed to excite the small intestine" by Nasset's method of assay (60), and Sonnenschein, Grossman, and Ivy (61) have recently reported that secretin is probably not the agent in intestinal extracts that stimulates the glands of Brunner in the duodenum

SECRETIN TEST FOR PANCREATIC FUNCTION

Early attempts (62, 63) to use secretin as a test for pancreatic function proved unsatisfactory because of inadequate methods of collecting the juice or the toxicity of the secretin preparations When Pancreotest became available through the cooperation of the Hammarsten group (20) with a Swedish manufacturer,* about 1936, Agren, Lagerlöf and Berglund (64) undertook to develop adequate methods for the estimation of pancreatic function in patients In order to secure pancreatic juice as free as possible from contamination with gastric juice they collected the gastric and duodenal contents separately through a special two-lumen tube so placed that one lumen opened into the pyloric portion of the stomach and the other into the duodenum After a control period they injected intra-

*Astra Sodertalje Sweden A domestic secretin preparation (Friedman 23) is now available from Wyeth Incorporated

erated into the blood from the living intestine and nothing to suggest the conditions under which it might function In the experiments of Farrell and Ivy (8) with the transplanted pancreas there were no significant differences in the enzyme content of the secretion whether they used HCl or various foods as stimuli even though some of the foods which they used have a powerful ecbolic action on the pancreas when it has its normal nerve supply This result may be interpreted as indicating that the ecbolic effect of food is not demonstrable in the totally denervated pancreas and is not, therefore, dependent on a hormonal mechanism The action of all the known ecbolic stimuli is suppressed by atropine (Chapter V) which is said not to modify the action of pancreozymin Until these aspects of the situation are clarified it will be necessary to reserve judgment with respect to the status of pancreozymin as a new hormone

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consequently the bile secreted during a test is normally stored in the gall bladder and does not appear in the duodenal contents If the gall bladder has been removed or if it is so diseased as to be nonfunctional the secreted bile is poured into the duodenum and appears in all the samples collected Three types of response are recognized (1) no bile in any sample - indicates obstruction in the biliary system,* (2) some but not all samples are free of bile or contain only traces--a normal response, (3) all samples heavily colored with bile--indicates a non-functioning gall bladder The results interpreted in this way show good correlation with the clinical and x-ray findings (72)

PANCREOZYMIN

It has long been known that crude secretin stimulates the pancreas to secrete juice with a greater concentration of enzymes than that obtained with purified secretin A fraction thought to be responsible for the ecbolic action of crude secretin was prepared from alcoholic extracts of the intestinal mucosa by Harper and Raper (73) in 1943 and given the name pancreozymin Their preparation did not stimulate the flow of pancreatic juice but in the presence of secretin it increased the output of enzymes Later Greengard and others (74) reported separating a similar material from their crude secretin by means of aniline precipitation Their product, like secretin, lost its activity on incubation with blood serum

It is easy to believe that pancreozymin is a specific hormone which, by its ecbolic action, complements the hydrelatic effect of secretin However, there is as yet no evidence that pancreozymin is lib-

*This response in the absence of associated jaundice is interpreted as normal

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Chapter VII

THE FUNCTIONAL INNERVATION OF THE PANCREAS

PROBABLY the first published observations clearly indicating the existence of secretory* nerves for the pancreas were those of Heidenhain (1, 2) although previous work (Bernard, Bernstein and others, for references see Babkin, 3) had indicated that pancreatic function was subject to nervous influences Heidenhain observed that electrical stimulation of the spinal cord often initiated pancreatic secretion or increased the flow of juice if the pancreas was already secreting He also made the important observation that the juice secreted in response to nerve stimulation contained more solids than the spontaneous secretion Heidenhain considered that the extrinsic nerves of the pancreas did not act as direct secretory nerves but were regulatory in their function like the nerves of the heart

It was not until Pavlov (4) devised his unique method for demonstrating the function of the vagus nerves in chronic animals that a clearer understanding of the extrinsic innervation became possible He exposed one of the vagus nerves in a pancreatic fistula dog under anesthesia and after cutting it brought the peripheral portion into the wound directly under

*This word is used throughout in the usual sense of causing secretion and not with the special meaning ascribed to it by Heidenhain (See introduction Chapter V)

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the skin The wound was closed with sutures and the animal allowed to recover When the nerve was again exposed four to eight days later, this time without anesthesia, stimulation of the peripheral portion caused an abundant secretion of pancreatic juice The failure to obtain similar results in acute experiments was due, as Pavlov thought, to the presence of inhibitory fibers in the pancreatic branches of the vagus and to the depressant effects of the anesthesia and trauma associated with the recent operation His method had the added advantage that the cardio-inhibitory fibers, which degenerated earlier than the pancreatic fibers, lost their function during the interval between the preliminary operation and the experiment

Pavlov also devised a method for demonstrating the secretory function of the vagi in acute experiments In dogs under light chloroform anesthesia he severed the spinal cord below the medulla and continued the experiment without further anesthesia The exact procedure has been described by Babkin (3) Under these conditions electrical stimulation of the peripheral vagus caused the pancreas to secrete but only after a long latent period and often only after several periods of alternate stimulation and rest If the pancreas was already secreting, stimulation of the vagus interrupted the secretion momentarily These results were interpreted as further evidence that the vagi convey two kinds of fibers to the pancreas, excitatory and inhibitory

The presence of inhibitory fibers was further demonstrated by Popielski* (5a, b) who showed that stimulation of the peripheral vagus could inhibit the secretory activity induced by HCl in the intestine He reported that certain branches of the vagi in the thorax

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*I am indebted to Dr S A Kornarov for an English translation of Popielski s Russian thesis

and abdomen were purely inhibitory while others were purely excitatory In the latter category he described a nerve bundle associated with the artery and vein of the pancreas which elicited a response from the pancreas comparable to that of the submaxillary gland to stimulation of the chorda tympani The artery referred to is probably the superior pancreaticoduodenal (see Richins, Ref 16, Chapter I) We (6) have confirmed the presence of secretory fibers in the nerve accompanying this artery but did not obtain the spectacular results reported by Popielski

The results reported by Pavlov and his pupils have been confirmed in their more general aspects by all subsequent investigators who have repeated their experiments However, von Anrep (7) was unable to confirm Popielski's results with regard to the separate secretory and inhibitory branches of the vagi, he found that all branches that affected the pancreas contained both types of fibers His work is of particular interest because he offered an explanation for the inhibitory effect of vagus stimulation which is now generally accepted He noted that the pancreas increased in volume when the vagus nerves were stimulated provided there was "inhibition" of secretion but not if a free flow of juice occurred He concluded that during the inhibitory phase the juice was secreted but retained in the gland owing to contraction of the ducts Von Anrep's hypothesis was later confirmed by Korovitsky (8) to the extent of proving that stimulation of the vagi causes contraction of the larger ducts Taking advantage of the fact that the pancreatic ducts anastomose within the gland, he caused fluid to flow into the duct system through one duct and out through the other The flow stopped when the vagus nerve was stimulated or pilocarpine administered but began again when atropine was given

Other methods than those described by Pavlov have since proved adequate for the study of the pancreatic

vagus in anesthetized animals Babkin (9) found that under chloralose anesthesia stimulation of the vagi below the heart increased pancreatic secretion in dogs with the central nervous system intact Previous section of the splanchnics had a favorable effect on the results In a series of experiments on dogs we (6) have been able to confirm Babkin's findings with regard to the favorable influence of chloralose anesthesia, especially when combined with urethane narcosis

Our experiments were undertaken primarily to test the effect of degeneration of one vagus on the secretory response to stimulation of the other nerve In one-third of the animals the right vagus was cut 10 to 21 days before the experiments These animals gave somewhat better results than the controls but the difference was not great enough to be conclusive We did not usually encounter the long latent periods mentioned in Pavlov's work nor the frequent failure of response to the first few stimulations although these manifestations of inhibition were evident in some of our animals

We found that animals with food residues in the stomach secreted better in response to stimulation of the vagus nerves than did starved animals Ligature of the pylorus did not alter this relationship (Fig 19 and 20) Although this latter fact proves that in most instances gastric HCl could not have been responsible for the individual bouts of secretion that occurred during stimulation of the vagus nerves, the evidence indicates that the presence of an accessory stimulus to secretion such as might accompany the occasional passage of gastric contents into the duodenum is important if not essential for the type of vigorous secretory response that we observed For example, in many experiments closure of the pylorus with a submucous ligature did not at once greatly modify the effect of the vagus on secretion but after a few stimulations the response to each became less (Fig 19) and

finally ceased altogether After an effective dose of secretin had been given and its immediate effects had passed off another series of secretory responses to stimulation of the vagus could be obtained The result accords with the observation of Gayet and Guillaumie (11a) who found that stimulation of the vagus nerves caused a several-fold increase in the response of the pancreas to secretin An example of synergism between secretin and the action of the vagus is shown in Fig 20 taken from one of our experiments

Evidently one effect of the vagi is to augment the action of other stimuli, nevertheless the nerves are capable under some circumstances of increasing pancreatic secretion independently Nakagawa and his co-workers (12) and later Crittenden and Ivy (13) observed that stimulation of the vagi elicited pancreatic secretion in dogs after removal of the entire small intestine The latter observed continuous secretion in the absence of the secretin mechanism and also demonstrated a secretory response to sham feeding in enterectomized animals

PROPERTIES OF PANCREATIC JUICE SECRETED IN RESPONSE TO STIMULATION OF THE VAGUS

All who have studied the problem agree that pancreatic juice secreted during stimulation of the vagus nerves or during the action of parasympathomimetic drugs contains a greater concentration of enzymes than that secreted during the action of secretin or of HCl in the intestine For example, Babkin and Savich (17) found that when acid was the stimulus the pancreatic juice contained 1 67 per cent solids, whereas stimulation of the vagus yielded a juice with a solid content of 7 43 per cent The increase in solids is entirely in the organic fraction and signifies an increase in enzyme content as shown in Table VIII (Savich, 15) The effect of the vagi on the pancreas

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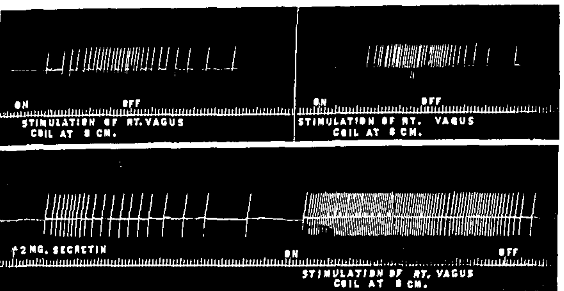


Figure 20 Synergistic action of secretin and vagus stimulation Kymographic record of pancreatic secretion in drops (vertical lines) induced by stimulation of the right vagus before (upper record) and after (lower record right) giving secretin *The effect of the secretin alone* is shown in the lower record to the left The upper record was made with the pylorus open but it was ligated before the lower record was made Sixteen kilo dog with a full stomach chloralose urethane anesthesia Time (lower line in each record) in 10 second intervals

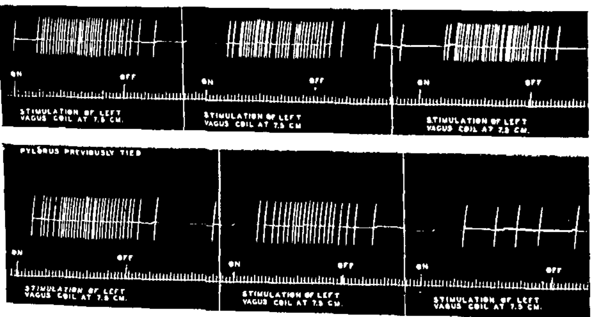


Figure 19 - Effect of ligating the pylorus on the response of the pancreas to stimulation of the vagus nerve Kymographic record of pancreatic secretion in drops (vertical lines) secreted in response to stimulation of the left vagus The upper record shows the results of three successive stimulations before ligating the pylorus Lower record shows the results of first third and seventh stimulations after placing a submucous ligature at the pylorus Eight kilo dog chloralose-urethane anesthesia right vagus degenerated time (lower line in each record) in 10 second intervals

even if they did they would not warrant the conclusion that the juice contained only active trypsin Babkin (3, p 469), who made a more thorough study of the problem, concluded that the amount of initially active trypsin in the juice depends on the total amount of enzyme present rather than on the method of stimulation Gayet and Guillaumie (11b) and Guillaumie (10) reached a similar conclusion The latter reported that the tryptic activity before activation with enterokinase was relatively no greater in "vagus juice" than in "secretin juice" if the difference in total enzyme content of the juice was taken into account

SPLANCHNIC SECRETORY FIBERS

Soon after the discovery of the secretory fibers in the vagus nerves Kudrevezki (18) demonstrated the presence of fibers with a similar function in the splanchnic nerves of dogs Here also inhibitory effects interfered with the secretion but this difficulty was overcome by Pavlov's method of partial degeneration of the nerves or by using mechanical stimuli Savich (15) was able to obtain secretion in some dogs by direct tetanization of the splanchnics without previous degeneration Baxter (19) demonstrated the presence in rabbits of secretory fibers in the splanchnic nerves, and Babkin, Hebb and Sergeyeva (20) studied them in cats as well as in dogs The last named investigators found that the secretion in cats was inhibited during the actual stimulation but increased beyond the control rate after cessation of the electrical stimulus They also confirmed the previous observation of Kuré (21) that the secretory pathway was not interrupted by painting the celiac ganglion with nicotine In fact this procedure increased the effect so that secretion was increased during the actual stimulation as well as later

In the experiments just described (20) the inhibi-

is, therefore, primarily ecbolic with only a moderate hydrelatic action, indeed in the cat only the ecbolic action can be demonstrated (8, 16)

The statement is often seen in textbooks that pancreatic juice secreted during stimulation of the vagus nerves contains active trypsin but the juice obtained *with secretin does not* This statement is based on

TABLE VIII

Retabulation of some data from Savich (15) showing secretion of active trypsin by the pancreas as well as an increase in total tryptic activity on stimulation of the vagus

Stimulus	Relative Rate of Secretion	Digestion of Egg White (Mett)	
		Pancreatic Juice	*Juice + Duodenal Contents*
		mm	mm
HCl	12	0 0	4 9
HCl	13	0 0	4 5
Vagus	3	1 7	7 2
Vagus	3	1 4	7 1
Vagus	7	0 6	6 8
Vagus	9	trace	6 2
Secretin	28	0 0	5 4

the observations of Savich (15) on pancreatic juice collected in acute experiments on dogs with the duct cannulated His trypsin determinations were made by the Mett method which requires prolonged incubation of the juice with the substrate Under these circumstances he found that some of the samples obtained during stimulation of the vagus nerves caused a certain amount of digestion of egg-white without addition of enterokinase from the intestine Even these samples showed much greater *proteolytic activity when* mixed with duodenal contents (Table VIII) *These experiments merely prove* that the trypsin in some samples was active at some time during the period of incubation with the substrate They do not necessarily show that it was active at the start (see Chap III) but

even if they did they would not warrant the conclusion that the juice contained only active trypsin Babkin (3, p 469), who made a more thorough study of the problem, concluded that the amount of initially active trypsin in the juice depends on the total amount of enzyme present rather than on the method of stimulation Gayet and Guillaumie (11b) and Guillaumie (10) reached a similar conclusion The latter reported that the tryptic activity before activation with enterokinase was relatively no greater in "vagus juice" than in "secretin juice" if the difference in total enzyme content of the juice was taken into account

SPLANCHNIC SECRETORY FIBERS

Soon after the discovery of the secretory fibers in the vagus nerves Kudrevezki (18) demonstrated the presence of fibers with a similar function in the splanchnic nerves of dogs Here also inhibitory effects interfered with the secretion but this difficulty was overcome by Pavlov's method of partial degeneration of the nerves or by using mechanical stimuli Savich (15) was able to obtain secretion in some dogs by direct tetanization of the splanchnics without previous degeneration Baxter (19) demonstrated the presence in rabbits of secretory fibers in the splanchnic nerves, and Babkin, Hebb and Sergeyeva (20) studied them in cats as well as in dogs The last named investigators found that the secretion in cats was inhibited during the actual stimulation but increased beyond the control rate after cessation of the electrical stimulus They also confirmed the previous observation of Kure (21) that the secretory pathway was not interrupted by painting the celiac ganglion with nicotine In fact this procedure increased the effect so that secretion was increased during the actual stimulation as well as later

In the experiments just described (20) the inhibi-

tory effects were less pronounced if the adrenal gland on the stimulated side was ligated Most observers have reported that epinephrin inhibits pancreatic secretion but Goldstein (22), using a perfused pancreas, found that if the vasoconstriction was compensated by an increase in perfusion pressure epinephrine increased the enzyme output Certainly the inhibitory effects of epinephrine as well as those associated with stimulation of the splanchnic nerves are due in part to vasoconstriction On the other hand Harper and Vass (23), because they observed an increase in enzyme output after cutting the splanchnic nerves in cats, concluded that these nerves contain specific inhibitory fibers for the acinous cells Babkin (9, 20) has suggested that contraction of the ducts may also be a factor in the inhibition

Most observers agree that the splanchnic secretory fibers are paralyzed by atropine Babkin and coworkers (20) provided direct evidence that they are cholinergic by detecting an increase in acetylcholine in the pancreatic vein following splanchnic stimulation The pancreatic juice secreted during stimulation of the splanchnic nerves, like that obtained during vagus stimulation, is rich in organic matter and contains a high concentration of enzymes (15) Generally speaking the splanchnics are less effective than the vagi as either ecbolic or hydrelatic agents

Kuntz (35) has recently confirmed the presence of sympathetic secretory and inhibitory elements for the pancreas in the celiac plexus The secretory fibers he finds, as have others, are cholinergic and the inhibitory elements are adrenergic Since he holds with Richins (36) that all sympathetic fibers to the pancreas end in relation to blood vessels he concludes that the secretory and inhibitory effects are exclusively referable to tonic changes in the vascular bed

Stimulation of either the splanchnics or the vagi causes significant changes in the microscopic appear-

ance of the acinous cells, due chiefly to dissolution of zymogen granules A fuller discussion of these changes will be given in the next chapter

INFLUENCE OF THE EXTRINSIC NERVES ON PANCREATIC BLOOD FLOW

Pavlov (4), Gottlieb (24) and more recently Babkin (9) and many others have reported that the secretory activity of the pancreas is greatly influenced by changes in its blood supply Conditions which decrease the blood flow through the gland diminish secretion while agents that increase the blood flow tend to augment secretory activity (25, 26) Bernard, and Kühne and Lea (see Heidenhain (2)) observed local vasodilation in the pancreas during active secretion Since the extrinsic nerves of the pancreas supply not *only the secretory elements but also the pancreatic* arteries, a part of their influence may be attributed to changes in blood flow through the gland

Following the early work of François-Franck and Hallion (27) there has been general agreement that the splanchnic nerves contain vasoconstrictor fibers for the pancreas By analogy with the vasomotor supply of other abdominal organs it may be assumed that they also convey vasodilators but these appear not to have been demonstrated specifically for the pancreas

Stimulation of the vagi has generally been found to increase the blood flow through the pancreas but the evidence that this is due to the action of vasodilator fibers is not wholly satisfactory François-Franck and Hallion (27) enclosed the pancreas in a plethysmograph and observed an increase in volume of the gland when the vagus fibers along the esophagus were stimulated, they assumed that the increase in volume was due to vasodilation It will be recalled that von Anrep (7) obtained a similar result but attributed it to an accumulation of secretion within the gland He

stated that the vagi were without effect on the pancreatic vessels Gayet and Guillaumie (28) enclosed the gland in a plethysmograph and at the same time measured blood flow with a "hemorheometer," a modification of Ludwig's stromuhr They found that the increase in volume during stimulation of the vagus nerves was associated with an increase in blood flow and had little relation to retention of secretion in the ducts

Bennett and Still (29) noted that any increase in pressure within the ducts caused an increase in blood flow as measured by an automatic mechanical stromuhr in the pancreaticoduodenal vein Their observation makes it almost impossible to prove the existence of a direct vasodilator action of the vagus nerves since an increase in intraductal pressure is almost certain to follow stimulation of fibers which both augment secretion and contract the ducts An apparently free flow of juice from the ducts does not, of course, prove that it is not flowing under increased pressure The point is largely academic since an increase in blood flow, however it is brought about, may be expected to favor secretory activity

Acid in the duodenum (30) and also secretin (29, 31) increase the blood flow through the pancreas but the mechanism is in doubt Only in the case of duodenal stimulation is there any probability that vasodilator reflexes are involved The effect of secretin may be attributed to either an increase in pressure within the ducts (29) or the action of local metabolites

REGULATION OF PANCREATIC SECRETION

It is evident from the data reviewed in Chapter V that all the individual food substances which serve as pancreatic stimuli excite secretion of pancreatic juice in which enzymes are present in greater concentration than in the juice secreted in response to acid or

secretin. The same is true of the secretion produced in the usual course of digestion (2, 3) The ordinary functional activity of the pancreas, therefore, involves reactions to ecbolic stimuli not accounted for by the secretin mechanism. The extrinsic innervation could provide such stimuli but the extent to which it does is a matter for experimental determination

Cutting the extrinsic nerves has surprisingly little effect on pancreatic function No observable loss of function results from division of the splanchnic nerves After the vagi are cut the cephalic phase of secretion is, of course, lacking but the ecbolic effect of meals is still present though definitely less than normal (32) In contrast, paralytic doses of atropine profoundly depress the secretion of enzymes in response to all types of alimentary stimuli If any conclusion can be drawn from these facts it is that there is more to the innervation of the pancreas than is comprised in the vagus and splanchnic fibers This conclusion accords with the situation in other abdominal organs in which the innervation is much better understood, specifically the stomach and small intestine Carlson (33) has compared the extrinsic nerves that supply these organs to association fibers between reflex centers and there is no doubt that the viscera mentioned continue to respond reflexly to local stimuli (34) in the absence of the extrinsic innervation

The earliest concepts of the innervation of the pancreas comprised the idea of local reflex mechanisms (5c) but these were generally forgotten after the discovery of secretin It is only recently that we have come to realize that the secretin hypothesis explains practically nothing except the response of the pancreas to acid and that acid is only one of many stimuli involved in the regulation of pancreatic function. The ecbolic effects of food and the relatively satisfactory functioning of the pancreas in achlorhydrics are not satisfactorily explained by the secretin hypothesis

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nor is the hydrelatic effect of food in those considerable intervals during digestion in which the intestinal acidity is too low to serve as a pancreatic stimulus In so far as these functions persist in the absence of the extrinsic innervation they may be due to local reflexes or to humoral agents Among the possible humoral agents with an ecbolic action, pancreozymin and blood glucose are the more promising from the standpoint of available experimental data

Local reflex paths, if they exist, probably consist of connections between the intrinsic ganglia of the pancreas and the enteric nerve plexus The developmental relations in the embryo strongly suggest the possibility of such connections but neither their presence nor their absence appears to have been demonstrated by morphological methods The possibility of intestino-pancreatic reflexes by way of the sympathetic ganglia may also be considered Should local reflex paths ultimately be demonstrated the extrinsic nerves could be considered not only as efferent paths in long reflex arcs but also as association fibers, to facilitate (or inhibit) local reflexes The former function is evident during the cephalic phase of pancreatic secretion and the latter may manifest itself during the intestinal phase by increased responsiveness of the pancreas to intestinal stimuli

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Chapter VIII

THE MECHANISM OF PANCREATIC SECRETION

1 SECRETION OF INORGANIC CONSTITUENTS

IN spite of conclusive evidence that secretion of enzymes by the pancreas is controlled separately from secretion of fluid and bicarbonate, it is commonly believed that the acinous cells elaborate the entire secretion This belief is based on the absence of other suitably situated cellular elements having the structural features commonly associated with secretory cells Also there is some evidence that fluid passes through the acinous cells when the pancreas is secreting even in the absence of specific ecbolic stimuli For example, Babkin, Rubaschkin and Savich (1) studied the pancreas microscopically after acid stimulation and concluded that the minor changes observed in the acinous cells were due to the passage of fluid through the cells The presence of intracellular structures which have been interpreted as secretory canaliculi (Chap I) is also suggestive

On the other hand some recent work indicates the possibility of dissociation of functions so complete as to demand a reexamination of the problem Lagerlöf (2) has reported that in many patients with pancreatitis the bicarbonate secretion may be normal even though the enzyme output is reduced, for example, of 32 patients with this disease 21 secreted a normal amount of bicarbonate when given secretin but only 12

produced a normal concentration of enzymes In similar patients Friedman and Snape (3) found some whose pancreatic juice was wholly without tryptic activity but who secreted a normal amount of alkaline fluid in response to secretin In a series of experiments on dogs Grossman and Ivy (4) observed the opposite type of dissociation of functions In animals that were made diabetic with alloxan the concentration of enzymes in the pancreatic juice remained normal but the usual threshold dose of secretin failed to cause secretion of fluid The acinous cells in the alloxan-treated animals retained a normal appearance but the cells of the intralobular ducts showed vacuolization The authors suggested the possibility that the duct cells participate in the elaboration of pancreatic juice and are, perhaps, the cells that respond to the action of secretin At present we do not know which pancreatic cells secrete bicarbonate

Source of bicarbonate Numerous efforts have been made to learn whether the bicarbonate of the pancreatic juice comes from the blood or from some other source within the gland but until very recently no convincing experiments have been reported The experiments of Still, Bennett and Scott (11) are often cited as evidence that much of the bicarbonate of the juice comes from the carbon dioxide produced by the oxidative metabolism of the cells These investigators measured the carbon dioxide and oxygen content of the arterial and venous blood of the pancreas and found that although the venous carbon dioxide fell, sometimes below arterial levels, during the early stages of secretion, it rose above resting levels if the secretion was prolonged

These observations prove that the total metabolic carbon dioxide is occasionally less but usually greater in amount than the total which appears as bicarbonate in the juice but they have no direct bearing on the source of bicarbonate in the juice The quantity

measured, the arteriovenous carbon dioxide difference, will always be equal to the total metabolic carbon dioxide minus that secreted in the juice The result will be the same whether the carbon dioxide of the juice is subtracted directly from the blood or from the metabolic carbon dioxide before it is added to the blood The way in which the metabolic carbon dioxide is divided between the juice and the blood cannot be calculated from these data nor can the amount of blood bicarbonate which is secreted in the juice

Montgomery and co-workers (5) found that radioactive sodium appeared in the juice within three minutes after it was injected into the blood, after fifteen minutes the concentration in the juice became equal to that in plasma and remained so throughout the experimental period Ball, Tucker and others (6) injected bicarbonate containing radioactive carbon into the blood while collecting pancreatic juice The radioactivity of the juice attained a level four to five times that of the blood, corresponding to the ratio of total bicarbonate in juice and blood This latter observation proves that the greater part of the bicarbonate of pancreatic juice comes from the blood

The oxygen consumption of the pancreas The knowledge that we have concerning the exchange of energy by the pancreas during secretion has been obtained in experiments in which secretin was used as a stimulus so that the work done was mainly concerned with secretion of fluid and bicarbonate Bancroft and Starling (9) measured the oxygen consumption of the pancreas at rest and during the action of secretin The oxygen consumption was high (0 05 cc /gm /min) in the resting gland and was increased about 30 per cent during the action of secretin Gerard and Still (10) saw about the same percentile increase in oxygen consumption (20 to 50% Av 30%) when secretin was applied to slices of pancreatic tissue in the Warburg apparatus Other tissues (except liver in one experiment) showed

no change in respiration when secretin was added With the gland in situ Still and others (8, 11) observed much larger increases in respiration (300% as indicated by CO_2 output) during the action of secretin Resting oxygen consumption in their experiments averaged 0 029 cc per gram per minute The increase in oxygen consumption after secretin persisted for as long as 30 minutes after the secretion had ceased, indicating accumulation of an oxygen debt during the period of activity The excess oxygen required over the amount supplied during active secretion amounted in some instances to 50 per cent of the total oxygen utilized Respiratory quotients of the pancreas ranged from 0 71 to 1 01 (resting average 0 87)

Secretory pressure in the pancreatic ducts The pressure developed when the pancreas is stimulated with the ducts occluded ranges around 300 mm H_2O (about 22 mm Hg) The reported results of several measurements are given in Table IX As Babkin (19)

TABLE IX

Secretory pressure in pancreatic ducts in mm H_2O

Observer	Dog	Cat	Rabbit	Monkey
Henry and Wollheim (13)			219 225	
Kuwschinski (14)	284 (21 mm Hg)			
Herring and Simpson (16)	313	303		277
Harms (17)	178 427			
Zucker Newburger and Berg (18a)	280 320			
Zucker Newburger and Berg (18b)	340 390			

has pointed out, the pressure that develops when the ducts are occluded is probably a measure, not of the secretory pressure as such, but of the ability of the finer ducts to retain fluid under pressure The pancreas becomes edematous when the ducts are occluded

(16), indicating penetration of the ducts by the secretion Experiments with injection of the ducts (Chap I) have shown that they are readily penetrated by injection fluids under fairly low pressure

Apparently no attempt has been made to determine *the influence, if any, of the protein content of the juice* on the secretory pressure This would be of interest because if a positive correlation were found it would suggest that the secretory pressure is really a measure of the colloid osmotic pressure of the pancreatic juice

The maximal pressures developed in the pancreatic ducts correspond rather closely to the maximal secretory pressures of the liver as measured in the bile ducts but Harms (17) found that when both pressures were measured simultaneously in the same animal the pressure in the pancreatic ducts was generally higher, by from 38 to 150 mm H_2O He found the biliary pressure higher only early in digestion when both pressures were low

Permeability of the pancreas Any discussion of permeability in connection with the pancreas must of necessity be in general terms We know only which substances appear in the juice when they are present in, or added to, the blood, in most instances we do not know through which of several possible cell groups they penetrate

Some or all of the pancreatic cells are sufficiently permeable to water and some electrolytes to maintain osmotic equilibrium with the blood (20, 21) Complete permeability to sodium (5, 20, 21) has been demonstrated Although the calcium concentration in the pancreatic juice is less than in the blood, the deficit is probably not due to relative impermeability of the cells to calcium but to the fact that much of the calcium of the blood is bound by protein and is not diffusible (Komarov and others, 7)

In contrast with its apparent free permeability to most cations the pancreas exhibits a high degree of

no change in respiration when secretin was added With the gland in situ Still and others (8, 11) observed much larger increases in respiration (300% as indicated by CO_2 output) during the action of secretin Resting oxygen consumption in their experiments averaged 0 029 cc per gram per minute The increase in oxygen consumption after secretin persisted for as long as 30 minutes after the secretion had ceased, indicating accumulation of an oxygen debt during the period of activity The excess oxygen required over the amount supplied during active secretion amounted in some instances to 50 per cent of the total oxygen utilized *Respiratory quotients of the pancreas* ranged from 0 71 to 1 01 (resting average 0 87)

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pressure In fact many agents that lower blood pressure augment the response to secretory stimuli, sometimes to a surprising extent (Fig. 21). This is particularly true of vasodilator substances such as peptone and nitrites which may increase the flow of blood even though the pressure falls Pavlov (15) has shown that the secretory pressure may exceed the blood pressure for a time when the blood pressure is lowered by venesection and the pancreas is stimulated through the vagi

The carbohydrate metabolism of the pancreas: Babkin (23) and his pupils have emphasized the importance of carbohydrate as a source of energy for the secretory work of the pancreas The possible relation of the blood sugar level to the output of pancreatic enzymes has been discussed briefly in Chapter V The pancreatic tissue contains glycogen and the amount can be increased by administration of glucose Insulin interferes with deposition of glycogen but does not of itself deplete the glycogen stores of the gland (24a) Miss Hebb (24b) found the pancreas slightly permeable to glucose in that varying amounts of sugar appeared in the pancreatic juice in the presence of hyperglycemia, with blood sugar levels between 100 and 200 mg per cent the sugar in the juice ranged from traces to 10 mg per cent Larger amounts, up to 100 mg per cent, appeared in the juice when the blood sugar levels were further increased She reported that stimulation of the vagus nerves decreased the amounts of sugar in the juice (see Babkin, (23) p 774) but the output was not affected by atropine (24b)

selectivity in the transfer of anions Only traces of phosphate are present in the juice and the chloride concentration is lower and the bicarbonate proportionally higher than in the blood Ball et al (6) as well as Hober (22) have attempted to explain these facts on the assumption that the pancreas is freely permeable to bicarbonate but less permeable to chloride and phosphate It is probably true that some portion of the secretory mechanism, acinous cells or ducts, is freely permeable to those substances which appear in equal concentration in the blood and pancreatic juice, but it is not so evident that differences in concentration of other substances can be fully explained by corresponding differences in permeability Davenport (12) has pointed out that this theory requires that the energy for secretion must come only from the blood pressure, and that there is not enough energy available from this source to provide for secretion of the inorganic constituents even when the pressure of the juice is low He calculates the energy available from the blood pressure as follows If the capillary pressure is 30 mm and the pressure of the juice zero so that the pressure falls through 30 mm Hg or 40 5 cm H_2O, for each liter (1000 gm) of juice secreted there is a decrease in potential energy of (40 5) (1000) gm cm One gm cm equals 2 34 x 10^{-5} calories The energy available from this source is therefore (40 5) (1000) (2 34 x 10^{-5}) = 0 95 gram-calories for each liter of juice secreted This value appears negligible when compared with the energy actually expended as indicated by the increase in oxygen consumption Davenport estimates the minimum amount of energy required to separate the inorganic constituents of one liter of pancreatic juice from the blood plasma to be of the order of 100 gram calories

Although the secretory activity of the pancreas is dependent on an adequate blood supply (See Chap VII), it is influenced more by blood flow than blood

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2 SYNTHESIS AND SECRETION OF ENZYMES

The production of enzymes by the pancreas involves two distinct processes, (1) synthesis within the gland and (2) discharge of enzymes into the secretion The first is believed to be a continuous process not subject

selectivity in the transfer of anions Only traces of phosphate are present in the juice and the chloride concentration is lower and the bicarbonate proportionally higher than in the blood Ball et al (6) as well as Hober (22) have attempted to explain these facts on the assumption that the pancreas is freely permeable to bicarbonate but less permeable to chloride and phosphate It is probably true that some portion of the secretory mechanism, acinous cells or ducts, is freely permeable to those substances which appear in equal concentration in the blood and pancreatic juice, but it is not so evident that differences in concentration of other substances can be fully explained by corresponding differences in permeability Davenport (12) has pointed out that this theory requires that the energy for secretion must come only from the blood pressure, and that there is not enough energy available from this source to provide for secretion of the inorganic constituents even when the pressure of the juice is low He calculates the energy available from the blood pressure as follows If the capillary pressure is 30 mm and the pressure of the juice zero so that the pressure falls through 30 mm Hg or 40 5 cm H_2O, for each liter (1000 gm) of juice secreted there is a decrease in potential energy of (40 5) (1000) gm cm One gm cm equals 2 34 x 10^{-5} calories The energy available from this source is therefore (40 5) (1000) (2 34 x 10^{-5}) = 0 95 gram-calories for each liter of juice secreted This value appears negligible when compared with the energy actually expended as indicated by the increase in oxygen consumption Davenport estimates the minimum amount of energy required to separate the inorganic constituents of one liter of pancreatic juice from the blood plasma to be of the order of 100 gram calories

Although the secretory activity of the pancreas is dependent on an adequate blood supply (See Chap VII), it is influenced more by blood flow than blood

pressure In fact many agents that lower blood pressure augment the response to secretory stimuli, sometimes to a surprising extent (Fig 21) This is particularly true of vasodilator substances such as peptone and nitrites which may increase the flow of blood even though the pressure falls Pavlov (15) has shown that the secretory pressure may exceed the blood pressure for a time when the blood pressure is lowered by venesection and the pancreas is stimulated through the vagi

The carbohydrate metabolism of the pancreas Babkin (23) and his pupils have emphasized the importance of carbohydrate as a source of energy for the secretory work of the pancreas The possible relation of the blood sugar level to the output of pancreatic enzymes has been discussed briefly in Chapter V The pancreatic tissue contains glycogen and the amount can be increased by administration of glucose Insulin interferes with deposition of glycogen but does not of itself deplete the glycogen stores of the gland (24a) Miss Hebb (24b) found the pancreas slightly permeable to glucose in that varying amounts of sugar appeared in the pancreatic juice in the presence of hyperglycemia, with blood sugar levels between 100 and 200 mg per cent the sugar in the juice ranged from traces to 10 mg per cent Larger amounts, up to 100 mg per cent, appeared in the juice when the blood sugar levels were further increased She reported that stimulation of the vagus nerves decreased the amounts of sugar in the juice (see Babkin, (23) p 774) but the output was not affected by atropine (24b)

2 SYNTHESIS AND SECRETION OF ENZYMES

The production of enzymes by the pancreas involves two distinct processes, (1) synthesis within the gland and (2) discharge of enzymes into the secretion The first is believed to be a continuous process not subject

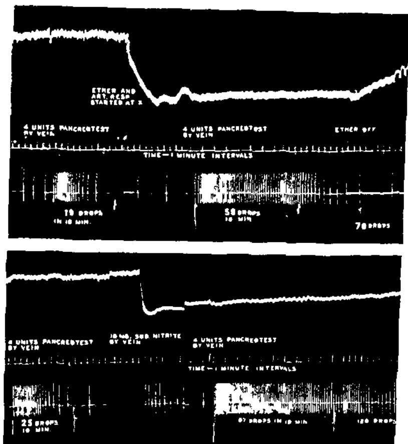


Figure 21

Effect of a fall in blood pressure on pancreat[illegible] -
tion Two kymographic records each showing
from above downw lood pressure (Hg
time in one minut , and drops of pan
as vertical lines cord respons
venous administr its (
are shown one b in
sure Note that
(vasodilation) eith
sodium nitrite (lowe
produced more than

to rapid change and, so far as we know, is influenced only by the laws of chemical equilibrium and by those factors in the internal environment that determine the rate of cellular metabolism in general It results in the elaboration and storage within the acinous cells of the characteristic pancreatic enzymes or their precursors Accumulation of zymogen granules within the cells is visible evidence of this process The second process, discharge of enzymes, is the characteristic response to ecbolic stimuli and involves the passage of stored material into the lumen of the acinus and eventually into the ducts This process, although ultimately dependent on synthesis, is independently controlled and may proceed so rapidly in response to specific stimuli that it outstrips synthesis leaving the cell depleted of visible zymogen material Secretion of enzymes will be considered first since more is known about it and the knowledge provides some essential background for the discussion of synthesis

Secretion of enzymes Much of what we know of the mechanisms involved in the discharge of enzymes by the acinous cells of the pancreas had been learned by study of the microscopic appearance of the gland before, and after secretion Heidenhain (25) studied stained sections of the pancreas of dogs in the fasting state and at various times after feeding He described the following changes in the appearance of the cells during and after secretion (digestion of a meal) as compared with the resting cells (1) The entire cell was reduced in size (2) The granular inner or apical zone was diminished in size more than the outer clear zone, the latter, although smaller, now occupied relatively more of the cell volume (3) The zymogen granules of the inner zone were smaller in size and fewer in number than in the resting cell and those that remained were congregated near the apical border of the cell Similar differences were observed between the resting and active cells of the rabbit's pancreas

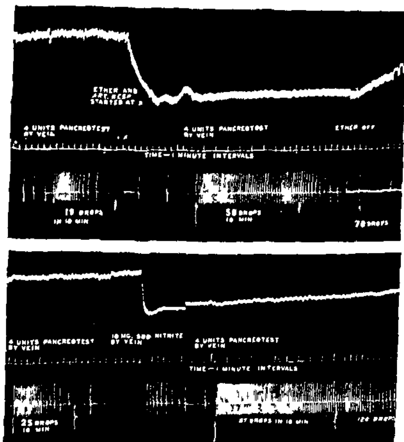


Figure 21

Effect of a fall in blood pressure on pancreatic secretion Two kymographic records each showing successively from above downward blood pressure (Hg manometer), time in one minute intervals and drops of pancreatic juice as vertical lines In each record two responses to intravenous administration of 4 units of secretin (Pancreotest) are shown, one before and one after a fall in blood pressure Note that after the blood pressure was reduced (vasodilation) either with ether (upper record) or with sodium nitrite (lower record) the same dose of secretin produced more than three times as much juice as before

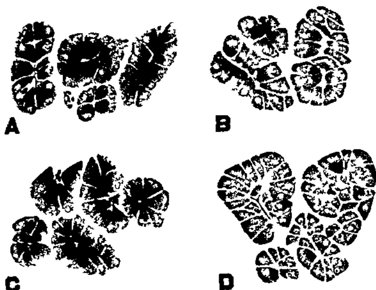


Figure 22

Pancreas of a dog A – in the fasting state B – after stimulation of the vagus nerves C – after secreting in response to acid D – after secreting in response to soap From Babkin Rubaschkin and Savich (1) Arch f mikr Anat 1909 Also Babkin Die äussere Sekretion der Verdauungsdrusen Second Edition Springer Berlin 1928

same time, secretion which took the same stain as the intracellular granules accumulated in the smaller ducts Stimulation of the splanchnic nerves had less effect than the vagi when the adrenal glands were intact but after ligation of the adrenal vessels the results more nearly approached those of vagus stimulation

Sergeyeva noticed that the acinous cells in the immediate vicinity of the islets contained more granules than the others, especially in the partly depleted gland,

but these were not correlated with phases of digestion since in this animal digestion is almost a continuous process Kühne and Lea (26) studied the pancreas of the living rabbit in situ and confirmed Heidenhain's observations with respect to the changes that occur in the cells during active secretion

Heidenhain (25) gave convincing proof that the zymogen granules are, in fact, the precursors of the enzymes which appear in the secretion He found that the enzyme content of the pancreas varied directly with the granule content of the cells Similarly, if the pancreas was exhausted by continuous secretion through a fistula the granules disappeared from the cells and enzymes were absent from the secretion as well as from extracts of the gland Others (1, 27, 28) have seen the granules pass directly into the secretion or dissolve in vacuoles which were later extruded into the lumen of the acinus

The changes in the appearance of the acinous cells during digestion may be taken as evidence that the pancreas is being acted upon by ecbolic stimuli The most thorough study of the effects of specific stimuli was made by Babkin, Rubaschkin and Savich (1) They found that stimulation of the vagus or splanchnic nerves caused a decrease in the number of zymogen granules in the acinous cells and migration of those that remained into the apical region of the cells Stimulation by means of soap had a similar effect but HCl caused only moderate changes and these were attributed to mechanical washing out of a few granules by the passage of fluid through the cells Some of their illustrations are reproduced in Fig 22

The experiments just referred to were done on dogs They were later repeated on cats by Sergeyeva (29) under Babkin's direction and quite similar results obtained *In the cat stimulation of the vagus alone* caused no visible secretion but the acinous cells nevertheless discharged their granules and, at the

Various considerations lead to the conclusion that the synthesis of enzymes proceeds within the cell until a dynamic equilibrium is established between the zymogen material and the various substances that enter into the reactions involved in its synthesis With this as a basic concept Langstroth, McRae and Komarov (33) made a combined experimental and mathematical analysis of the synthesis of enzymes based on the output of enzymes during the action of secretin Their study led them to the conclusion that the rate of synthesis was not influenced by the action of the parasympathetic nerves nor by the presence of secretin within the cells, that it is determined by the relative amounts of zymogen material (the end product of synthesis) and of various unknown substances supplied continuously to the gland cells from the blood

Hirsch (30) studied the regeneration of granules in the pancreas of living white mice by direct microscopic observation of the gland after the cells had been depleted by giving pilocarpine until they contained only a few granules New granules first appeared in the basal portion of the cell as tiny specks attached to the mitochondria They continued in this relation for only a short time, 10 - 17 minutes, and then became free and began a curious to and fro motion in the cytoplasm, meanwhile increasing in size They also increased in number at first but later the numbers decreased Whether or not the decrease in numbers was caused by fusion of particles the author did not decide After 1 1/2 to 3 hours the larger granules changed their type of motion and began to drift toward the apical zone of the cell where they eventually came to rest Here they grew still larger and changed their reaction to stains, finally becoming mature zymogen granules Only about two-thirds of the granules had matured in 10 - 11 hours but the author expressed the opinion that the cells would already have been capable of secreting in 3 1/2 hours Duthie (34) repeated and

and thus formed visible "halos" of darker staining cells around the islets Stimulation of the vagi brought these "halos" into contrast by causing depletion of the other cells, leaving those around the islets more or less in the resting condition Stimulation of the splanchnics with the adrenal vessels ligated depleted the "halo" cells as well as the others Some light may be cast on this phenomenon by the peculiar arrangement of the blood vessels in the vicinity of the islets which was mentioned in Chapter I There it was pointed out that the acinous cells surrounding the islets receive blood that has just previously passed through the sinusoids of the islets and may therefore contain more insulin than the systemic arterial blood that supplies the remainder of the gland These observations again suggest a close relation between the secretion of enzymes and the carbohydrate metabolism of the secreting cells as recently emphasized by Babkin (23)

The effects on the appearance of the acinous cells of stimulation of the vagus nerves may be duplicated by administration of parasympathomimetic drugs such as pilocarpine (27, 30), or choline chloride (29) The presence of peptone in the intestine has a similar effect (Chap V) and so does pancreozymin (31)

Synthesis of enzymes The first investigation of the restitution of zymogen material within the pancreatic cells following their depletion by active secretion was undertaken by Heidenhain (25) and by Grutzner (32) They studied the enzymatic activity of pancreatic extracts made from the glands of dogs that had been killed at various times after feeding As expected, the enzyme content of the glands decreased during the first six to ten hours, it then gradually increased until a near maximum was attained at the 14th to 16th hour after feeding Thereafter the trypsin remained constant, the amylase decreased somewhat but the lipase content continued to rise slowly until the 40th hour

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confirmed these observations He agreed that the granules appear first in the basal zone of the cell on or near the mitochondria and then migrate to the apical zone where they become enmeshed in the Golgi apparatus and come to rest He distinguished the immature from the mature granule by the fact that the former stains with vital red while the latter does not *Others have held that the zymogen granules appear* first in relation to the Golgi material For a discussion of this view see Babkin (23)

It maybe of interest in this connection to note that granules appear in the acinous cells of the developing pancreas as soon as the acini are formed, on the 17th or 18th day of development in the embryo rat (35)

Electrical changes in the pancreas The pancreas, like other tissue, exhibits a negative electrical potential relative to resting tissue when stimulated Von Anrep and Daly (36) placed two electrodes, one on the tail of the pancreas and the other within the pancreatic duct, in anesthetized cats and recorded the potential difference with a galvanometer During bouts of secretion induced by secretin the tail (acinous tissue) showed a negative potential relative to the duct Control experiments proved that these potentials were not caused by a fall in blood pressure nor by the flow of juice past the electrode in the duct

Hasama (37) used a unipolar method with a recording electrode on the pancreas and an indifferent electrode elsewhere on the body of the animal Stimulation of the vagus or splanchnic nerves or injection of secretin brought about a negative potential in the pancreas, relative to the body of the animal, which paralleled the secretion of pancreatic juice

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This Book

THE EXTERNAL SECRETION OF THE PANCREAS

By J EARL THOMAS, M D

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